# Einfluss CD4-positiver Lymphozyten auf die adipositasassoziierte Inflammation im murinen Fettgewebe

Dissertation
zur Erlangung des akademischen Grades
Dr. med.

an der Medizinischen Fakultät
der Universität Leipzig

eingereicht von:
Georg Friedrich Brinker
geb. am 13.11.1993 in Kiel

angefertigt am:
Institut für Anatomie
Medizinische Fakultät der Universität Leipzig

Betreuung durch:
Herrn Prof. Dr. med. Martin Gericke
Herrn Prof. Dr. med. Ingo Bechmann

Beschluss über die Verleihung des Doktorgrades vom:
24.11.2021

**Inhalt**

# 1 Einführung

## 1.1 Fettgewebe

Unser Wissen über Herkunft, Art und Physiologie des Fettgewebes hat sich seit der Mitte des letzten Jahrhunderts erheblich verändert, nicht zuletzt aufgrund der Erkenntnis, dass die krankhafte Expansion des Fettgewebes die weltweite Morbidität und Mortalität wesentlich beeinflusst. In Säugetieren gibt es prinzipiell zwei distinkte Formen von Fettgewebe, das weiße und das braune Fettgewebe, die sich vor allem in Form und Anzahl der Lipidtropfen und Mitochondrien der Fettzellen unterscheiden (Frontini et Cinti 2010).

Das weiße Fettgewebe besteht vorwiegend aus univakuolären Zellen, die unter Einlagerung von Lipiden bei einer positiven Stoffwechselbilanz eine Größe von bis zu 120 µm erreichen können (Welsch, 2014). Die zentrale Funktion dieses Fettgewebes ist die Gewährleistung der Energie- und Nährstoffhomöostase durch Speicherung und Abgabe von Lipiden (Rosen et Spiegelman 2014). Diese Fähigkeit ist ein phylogenetisch hochkonservierter Mechanismus (Zwick et al. 2018), der ausschließlich in Wirbeltieren von einem speziell dafür vorgesehenen Gewebe übernommen wird (Birsoy, Festuccia et Laplante 2013). Es wird angenommen, dass dieses Gewebe hauptsächlich mesodermalen Ursprungs ist, obwohl auch die Neuralleiste als Ursprung insbesondere für Adipozyten in der Kopfregion diskutiert wird (Billon, Monteiro et Dani 2008). Das weiße Fettgewebe ist im Menschen in verschiedenen Depots verteilt, wobei Verteilung und Funktionalität dieses Gewebes sehr heterogen sind und unter anderem von Geschlecht, Krankheit, Genetik und Alter abhängen (Tchkonia et al. 2013). Auch zwischen Mäusen und Menschen gibt es erhebliche Unterschiede (Rosen et Spiegelman 2014). In beiden Organismen werden jedoch ganz grundlegend viszerale, also um die inneren Organe gelegene, von subkutanen Depots unterschieden (Rosen et Spiegelman 2014). Das viszerale Fettgewebe befindet sich im Menschen vor allem omental, mesenterial, retroperitoneal, gonadal und perikardial (Zwick et al. 2018). Das subkutane weiße Fettgewebe befindet sich vor allem abdominell und gluteofemoral (Kwok, Lam et Xu 2016). Darüber hinaus gibt es auch diskretere, gewebeassoziierte Depots, wie zum Beispiel das dermale Fettgewebe, die phänotypisch und funktionell von anderen Depots abgrenzbar sind (Kruglikov et Scherer 2016). Funktionell dient das Fettgewebe neben seiner zentralen Funktion als Speicherfett, auch als Bau- oder Isolierfett (Welsch, 2014). Seit der Entdeckung von „Fettgewebe-Hormonen“, sogenannten Adipokinen, wie Leptin (Zhang et al. 1994), Adiponektin (Scherer et al. 1995) und Visfastin (Fukuhara et al. 2005), wird das Fettgewebe außerdem als endokrines Organ begriffen (Waki et Tontonoz 2007).

Das braune Fettgewebe unterscheidet sich in Funktionalität, Verteilung und Herkunft erheblich vom weißen Fettgewebe. Charakteristisch für dieses Fettgewebe ist vor allem die hohe Expression des *uncoupling protein 1* (UCP-1), ein Protein, welches die Atmungskette von der

ATP-Synthese entkoppelt und auf diesem Wege chemische in thermische Energie, in Form von Wärme, übersetzt (Cohen et Spiegelman 2015). Dieser Mechanismus begründet die zentrale Bedeutung des braunen Fettgewebes für die Thermoregulation (Cohen et Spiegelman 2015). Braune Adipozyten sind multivakuolär und reich an Mitochondrien und sind daher phänotypisch von weißen Fettzellen zu unterscheiden (Frontini et Cinti 2010). Weiterhin teilen sich braune Fettzellen einen Vorläufer mit Skelettmuskelzellen, der aber für die weiße Adipogenese unbedeutend ist (Seale et al. 2008). Depots von braunem Fettgewebe befinden sich vor allem zwischen den Schulterblättern und perirenal (Rosen et Spiegelman 2014), allerdings ist seit langem bekannt, dass in der Maus auch in weißem Fettgewebe UCP-1 produzierende, multivakuoläre Zellen auftreten können (Young, Arch et Ashwell 1984). Dieses Gewebe wird aufgrund seiner Ähnlichkeit zum braunen Fettgewebe als beiges Fettgewebe bezeichnet. Im Gegensatz zum braunen Fettgewebe besitzt es allerdings nur eine sehr geringe basale Expression von UCP-1, die aber durch Kälteexposition oder adrenerge Stimulation schnell hochreguliert werden kann (Wu et al. 2012). Es wurde lange angenommen, dass braunes Fettgewebe nur bei Kindern und kleineren Säugetieren auftritt, allerdings konnte mit Hilfe von modernen Bildgebungsmodalitäten nachgewiesen werden, dass auch erwachsene Menschen umgrenzte Depots von thermogenetisch aktiven Fettzellen besitzen (Virtanen et al. 2009; van Marken Lichtenbelt et al. 2009; Cypess et Kahn 2010). In seiner Gesamtheit ist das Fettgewebe also ein sehr heterogenes Organ mit je nach Art und Ort ganz unterschiedlichen Eigenschaften.

## 1.2 Adipositas und adipositasassoziierte Erkrankungen

Das weiße Fettgewebe ist ein äußerst plastisches Organ, dessen Anteil am Körpergewicht zwischen 5% und 60% variieren kann (Cawthorn, Scheller et MacDougald 2012). Diese Eigenschaft ermöglichte in den letzten Jahrzehnten einen globalen Anstieg der Prävalenz von Adipositas, also der krankhaften Vermehrung von Fettgewebe (Ng et al. 2014). Allein in Deutschland waren 2013 21,9% der Männer und 22,5% der Frauen adipös (Ng et al. 2014). 1980 hatte sich dieser Anteil noch auf 15,9% und 17,2% belaufen (Ng et al. 2014). Problematisch ist diese Entwicklung vor allem deshalb, da die Adipositas mit weiteren Komorbiditäten, wie Bluthochdruck (Fox et al. 2007) und Diabetes (Wang et al. 2005; Carey et al. 1997), aber auch verschiedenen Krebsentitäten (Bhaskaran et al. 2014), vergesellschaftet ist. In der Gesamtheit führt diese adipositasassoziierte Morbidität zu einem deutlich erhöhten kardiovaskulären Risiko (Kivimäki et al. 2017) und nachweislich auch zu einer erhöhten Mortalität (Di Angelantonio et al. 2016). Interessanterweise scheint vor allem die Expansion des viszeralen Fettgewebes die adipositasassoziierte Morbidität und Mortalität zu verantworten, wohingegen der Beitrag des subkutanen Fettgewebes weniger klar ist und sogar vorteilhafte Effekte diskutiert werden (Lee, Wu et Fried 2013). Insbesondere der Ausprägungsgrad einer

Insulinresistenz scheint eng mit der viszeralen Fettmasse zu korrelieren (Miyazaki et DeFronzo 2009; McLaughlin et al. 2011).

### 1.3 Adipositas, Inflammation und Insulinresistenz

Die Insulinresistenz ist ein wichtiges Kennzeichen der Manifestation eines Diabetes mellitus Typ 2. Pathophysiologisch werden bei dieser Erkrankung die insulinabhängigen Organe - Leber, Skelettmuskulatur und Fettgewebe - weniger empfänglich für die Aktivierung durch Insulin (Zatterale et al. 2019). Unter physiologischen Bedingungen steuert Insulin eine Reihe elementarer Prozesse, wie Zellwachstum und Differenzierung, sowie die Regulierung des Fett-, Protein- und Glukosestoffwechsels. So fördert Insulin als anaboles Hormon den Aufbau von Lipiden (Lipogenese), die Proteinbiosynthese und die Speicherung von Glukose als Glykogen und inhibiert im Gegenzug den Abbau dieser energiereichen Substrate. Insulin fördert außerdem die Aufnahme von Glukose in den Skelettmuskel und das Fettgewebe und hemmt die Glukoseproduktion (Gluconeogenese) in der Leber (Saltiel et Kahn 2001). Die Insulinresistenz führt nun zu einer Dysregulation dieser Prozesse und folglich zu Hyperglykämien und Hyperlipidämien (Saltiel et Kahn 2001). Über einen gewissen Zeitraum wird diese Dysregulation durch eine erhöhte Insulinproduktion in den β-Zellen des Pankreas kompensiert. Im weiteren Verlauf kommt es allerdings zu einer Erschöpfung der β-Zellen, einer persistenten Hyperglykämie und der Manifestation eines Diabetes mellitus Typ 2 (Shulman 2000).

Die molekularen Mechanismen, welche die Entstehung einer adipositasassoziierten Insulinresistenz begünstigen, sind Gegenstand aktueller Forschung. Momentan geht man davon aus, dass unter bestimmten genetischen Voraussetzungen im Verlauf einer hochkalorischen Diät parenchymale Zellen wie Adipozyten, Hepatozyten und Myozyten ein proinflammatorisches Programm von intrinsischen und extrinsischen Signalen initiieren (Lee, Wollam et Olefsky 2018b; Reilly et Saltiel 2017; Zatterale et al. 2019). Diese Signale induzieren proinflammatorische Zytokine (Zatterale et al. 2019; Lee, Wollam et Olefsky 2018b; Shoelson, Lee et Goldfine 2006) und führen damit letztendlich zu einer Akkumulation von Immunzellen, welche dann über autokrine und parakrine Mechanismen insbesondere im Fettgewebe eine Entzündungsreaktion etablieren und unterhalten (Lee, Wollam et Olefsky 2018b). Diese Inflammation beeinflusst den metabolischen Status der Parenchymzellen, indem insulinabhängige Signalwege gehemmt werden (Lee, Wollam et Olefsky 2018b). Die Übersetzung von kalorischem Exzess in eine chronische Gewebeinflammation wird mutmaßlich von einer Vielzahl unterschiedlicher Mechanismen initiiert, von denen einige hier kurz genannt werden sollen (Lee, Wollam et Olefsky 2018b; Reilly et Saltiel 2017):

Die Expansion des Fettgewebes unter der Zufuhr überschüssiger Kalorien führt zu einem vermehrten Zelltod von Adipozyten. Diese sterbenden Adipozyten werden von Makrophagen

ummauert. Morphologisch wird diese „Ummauerung“ als *crown-like structure* (CLS) beschrieben (Cinti et al. 2005; Murano et al. 2008). In diesen CLS befinden sich vor allem proinflammatorische Makrophagen (Lumeng et al. 2008; Spencer et al. 2010), die mit der Ausbildung einer Insulinresistenz in Verbindung gebracht werden (Patsouris et al. 2008). Im Verlauf der Ausdehnung des weißen Fettgewebes kommt es allerdings nicht nur zum Zelltod hypertropher Adipozyten, sondern auch zu einer relativen Minderperfusion und damit einer mangelnden Oxygenierung des Parenchyms (Sun, Kusminski et Scherer 2011; Trayhurn 2013). Diese Gewebehypoxie induziert durch die sogenannten *hypoxia-inducible factors* (HIFs) ein Genprogramm, welches die Angiogenese aber auch inflammatorische Signalwege aktiviert (Trayhurn 2013; Sun, Kusminski et Scherer 2011). In mehreren Studien konnte daher gezeigt werden, dass eine Inhibierung dieses Programms eine Abschwächung der Inflammation und eine Verbesserung der Insulinsensitivität nach sich zieht (Lee et al. 2014; Lee et al. 2011; Jiang et al. 2011). Daneben führt die Expansion der Adipozyten zu einem *remodelling* der extrazellulären Matrix (EZM) (Rutkowski, Stern et Scherer 2015). Dieses *remodelling* beinhaltet die abnorme Akkumulation bestimmter Kollagene (Kusminski, Bickel et Scherer 2016; Khan et al. 2009) und Khan et al. konnten zeigen, dass die genetische Deletion eines dieser Kollagene einhergeht mit einer Verbesserung der metabolischen und inflammatorischen Parameter (Khan et al. 2009). Auch *in vitro* ist die Insulinsensitivität der Adipozyten abhängig von der EZM-Dichte (Li et al. 2010). Diese Vorgänge werden begleitet von einem Anstieg freier Fettsäuren (Glass et Olefsky 2012). Insbesondere gesättigte Fettsäuren scheinen, über die indirekte Bindung an sogenannte *toll-like rezeptors* (TLRs), welche der Erkennung pathogener Strukturen dienen, einen proinflammatorischen Effekt zu vermitteln (Könner et Brüning 2011). Die Beobachtung, dass bestimmte TLRs in diabetischen Individuen überexprimiert werden, unterstreicht die Bedeutung dieses Mechanismus (Dasu et al. 2010).

Nicht zuletzt konnten auch mitochondriale Einflüsse und Stresssignale des endoplasmatischen Retikulums (ER) für die Initiierung proinflammatorischer Veränderungen im Rahmen der Adipositas verantwortlich gemacht werden (Zatterale et al. 2019). Mehrere Studien konnten mittlerweile etablieren, dass Adipositas mit einem Verlust mitochondrialer Funktionalität einhergeht und dass das Ausmaß der mitochondrialen Dysfunktion mit dem Grad der Inflammation und der Insulinresistenz korreliert (Heinonen et al. 2015; Qatanani et al. 2013; Yin et al. 2014). Neben der eingeschränkten Funktionalität der Mitochondrien in adipösen Individuen ist kürzlich außerdem gezeigt worden, dass der Transport von Mitochondrien aus Adipozyten in Makrophagen *in vivo* beeinträchtigt ist und dass dieser gestörte mitochondriale Transfer mutmaßlich die Entstehung einer Insulinresistenz mit beeinflusst (Brestoff et al. 2021). Auch die Integrität des endoplasmatischen Retikulums scheint bei der Adipositas beeinträchtigt zu sein, denn unter hochkalorischer Diät kommt es, insbesondere im Fettgewebe und im Leberparenchym, zu einer verstärkten Aktivierung spezieller Signalwege, welche zusammenfassend als ER-

Stress bezeichnet werden (Nakatani et al. 2005; Ozcan et al. 2004). Die Verbesserung des metabolischen Status nach genetischer oder pharmakologischer Inhibition der Stressantwort des ER hat die Bedeutung dieses Mechanismus in der Entstehung der adipositasinduzierten Insulinresistenz bekräftigt (Ozcan et al. 2004; Ozcan et al. 2006).

## 1.4 Fettgewebeentzündung

Proinflammatorische Veränderungen in Reaktion auf eine hochkalorische Diät sind nicht nur für die drei großen insulinabhängigen Organe Fettgewebe, Leber und Skelettmuskulatur, sondern auch für das Pankreas, das zentrale Nervensystem (ZNS) und den Gastrointestinaltrakt beschrieben worden (Lee, Wollam et Olefsky 2018a). Insbesondere im Fettgewebe aber induzieren diese proinflammatorischen Veränderungen eine Entzündungsreaktion (Weisberg et al. 2003). Diese Inflammation ist im viszeralen Fettgewebe besonders ausgeprägt (O'Rourke et al. 2009; Gastaldelli et al. 2002) und ist gekennzeichnet durch erhebliche quantitative und qualitative Veränderungen innerhalb des Immunzellreservoirs (Osborn et Olefsky 2012):

Unter physiologischen Bedingungen ist das Fettgewebe angereichert mit antiinflammatorischen Makrophagen (Lumeng, Bodzin et Saltiel 2007), regulatorischen T-Zellen (Feuerer et al. 2009), natürlichen Killer-T-Zellen (Lynch et al. 2012), angeborenen lymphoiden Zellen der Gruppe 2 (Brestoff et al. 2015) und eosinophilen Granulozyten (Wu et al. 2011). Im hyperkalorischen Zustand kommt es zu einer relativen Abnahme dieser Zellen und im Gegenzug zu einer relativen Zunahme proinflammatorischer Makrophagen (Lumeng, Bodzin et Saltiel 2007), Mastzellen (Liu et al. 2009) und neutrophilen Granulozyten (Talukdar et al. 2012). B-Zellen und T-Zellen sind auch im Fettgewebe schlanker Individuen enthalten, verändern allerdings unter einer hochkalorischen Diät ihren Phänotyp erheblich (Winer et al. 2009; Nishimura et al. 2009; Winer et al. 2011). Die diätabhängige Dynamik und Aktivität all dieser Zellpopulationen ist mittelbar mit Veränderungen der Insulinresistenz assoziiert.

## 1.5 Fettgewebemakrophagen

Die Makrophagen machen den größten Anteil der Immunzellen im Fettgewebe adipöser Individuen aus (Weisberg et al. 2003; Xu et al. 2003). So sind im Fettgewebe dünner Mäuse etwa 10% der Stromazellen Makrophagen. In hochkalorisch ernährten Mäusen steigt dieser Anteil bis auf über 50% (Weisberg et al. 2003). Makrophagen reagieren auf unterschiedliche Stimuli in ihrem Mikromilieu mit einer Veränderung ihres Phänotyps (man spricht auch von Aktivierung oder Polarisierung). Anhand eines weit verbreiteten Klassifizierungssystems, dem M1/M2-Paradigma, existieren diese Phänotypen in einem Kontinuum, innerhalb dessen im Grundsatz zwei Phänotypen unterschieden werden: Klassisch-aktivierte oder M1-Makrophagen und

alternativ-aktivierte oder M2-Makrophagen (Gordon 2003; Mantovani et al. 2004). M1-Makrophagen werden durch proinflammatorische Zytokine wie Interferon-γ (IFNg) und Tumornekrosefaktor α (TNFα) induziert und exprimieren CD11c auf ihrer Oberfläche. Demgegenüber exprimieren die sogenannten M2-Makrophagen die Oberflächenproteine CD206 und CD301 und werden durch die Zytokine Interleukin 4 (IL-4) und Interleukin 13 (IL-13) aktiviert (Martinez et Gordon 2014). Mittlerweile ist bekannt, dass dieses Schema zur Beschreibung der Komplexität und Dynamik der Gewebemakrophagen *in vivo* nicht hinreichend (Martinez et Gordon 2014; Russo et Lumeng 2018) und insbesondere auch nicht ohne Weiteres vom Mausmodell auf den Menschen übertragbar ist (Russo et Lumeng 2018). Im Mausmodell ist dieses Paradigma allerdings nützlich, insbesondere da gezeigt werden konnte, dass CD11c-positive und CD206-negative Makrophagen eine Verstärkung sowohl der Fettgewebeinflammation als auch der Insulinresistenz bedingen (Nguyen et al. 2007; Patsouris et al. 2008). Im Kontext der Fettgewebeinflammation unter einer hochkalorischen Diät kommt es zu einem Anstieg beider Phänotypen, verhältnismäßig aber zu einem "*phenotypic switch*" zugunsten der proinflammatorischen M1-Makrophagen (Lumeng, Bodzin et Saltiel 2007; Lumeng et al. 2008).

## 1.6 Ursprung von Makrophagen

### 1.6.1 Rekrutierung von Monozyten aus dem Blut

Bislang wurde angenommen, dass der Anstieg von Makrophagen im Fettgewebe die alleinige Folge einer vermehrten Rekrutierung von Monozyten sei (Nishimura et al. 2009). Diese Vermutung begründete sich in dem von van Furth et al. in den späten Sechzigern aufgestellten mononukleären Phagozytensystem (MPS). Van Furth et al. postulierten ein lineares System, in dem nur hämatopoetische Stammzellen zu Makrophagen differenzieren können und geweberesidente Makrophagenpopulationen durch die kontinuierliche Rekrutierung monozytärer Vorläuferzellen unterhalten werden (van Furth et Cohn 1968; van Furth et al. 1972). Die Anwendung von *fate mapping*-Techniken erbrachte jedoch den Nachweis einer weiteren Zelllinie von Makrophagen, die in der Embryonalentwicklung der Maus bereits vor den hämatopoetischen Stammzellen im Dottersack auftritt (Schulz et al. 2012) und völlig unabhängig von der primitiven Hämatopoese zu gewebespezifischen Makrophagen heranreift (Davies et al. 2013). Aus dieser Zelllinie gehen etwa die Mikroglia und die Langerhans-Zellen der Haut hauptsächlich hervor (Davies et al. 2013). Auch im Fettgewebe wurde eine Population von geweberesidenten Makrophagen beschrieben, die ihren Ursprung im Dottersack hat (Hassnain Waqas et al. 2017).

### 1.6.2 Makrophagenproliferation

Viele dieser Makrophagenpopulationen können zudem im *steady state* völlig unabhängig von Monozytenrekrutierung existieren. Dieser „Selbsterhalt“ geweberesidenter Makrophagen wird durch lokale Proliferation ermöglicht (Hashimoto et al. 2013). Auch Makrophagen des Fettgewebes besitzen die inhärente Fähigkeit zu proliferieren und interessanterweise ist der Anteil proliferierender Makrophagen im Fettgewebe adipöser Mäuse drastisch erhöht (Amano et al. 2014; Haase et al. 2014; Bourlier et al. 2008). Zheng et al. konnten durch Generierung Knochenmark-chimärer Mäuse zeigen, dass die Akkumulation von Fettgewebemakrophagen zweizeitig verläuft: Die Proliferation scheint vor allem im frühen Stadium und die Rekrutierung von Monozyten im späteren Verlauf der Adipositas wichtig zu sein (Zheng et al. 2016).

## 1.7 Regulation der Makrophagenakkumulation im Fettgewebe

Die Akkumulation von Makrophagen im Fettgewebe bedarf chemotaktischer Signale (Chawla, Nguyen et Goh 2011). Eine entscheidende Rolle wird dem Chemokinrezeptor *C-C chemokine receptor type 2* (CCR2) und seinem Liganden dem *monocyte chemoattractant protein-1* (MCP-1) zugeschrieben. Der Mangel an CCR2 führt zu einem reduzierten Anstieg von Makrophagen (Weisberg et al. 2006), die transgene Überexpression des entsprechenden Liganden wiederum zu einer verstärkten Makrophagenakkumulation (Kanda et al. 2006). Ganz ähnlich konnte auch für das Zytokin Osteopontin (OPN) eine Bedeutung für die Makrophagenakkumulation etabliert werden, da sowohl die Neutralisierung von OPN *in vivo,* als auch die genetisch induzierte OPN-Defizienz eine Verringerung der Makrophagenakkumulation erreichten (Kiefer et al. 2010; Nomiyama et al. 2007). Weiterhin wird angenommen, dass auch von den Adipozyten freigesetzte Fettsäuren lokal die Akkumulation von Makrophagen fördern, da im Fettgewebe von Mäusen ohne Adipozyten-Triglyceridlipase eine Verringerung der Makrophagenakkumulation festgestellt wurde (Kosteli et al. 2010). In diesem Sinne ist auch die Beobachtung zu verstehen, dass Mäuse ohne das Protein *CD5 antigen-like* eine verringerte Makrophageninfiltration ihrer Fettdepots aufweisen, da dieser endogene Faktor in Adipozyten die Lipolyse induziert (Kurokawa et al. 2011).

Die Proliferation von Fettgewebemakrophagen scheint ebenfalls durch die Zytokine MCP-1 und OPN beeinflusst zu werden (Amano et al. 2014; Tardelli et al. 2016). Letzteres wird im Fettgewebe adipöser Individuen vor allem von CD4-positiven T-Zellen exprimiert (Shirakawa et al. 2016). Durch Untersuchungen anderer Gewebearten ist außerdem bekannt, dass die Proliferation von Makrophagen unter dem Einfluss des T-Zell-assoziierten Zytokins Interleukin 4 (IL-4) reguliert wird (Jenkins et al. 2011). Zellkulturstudien legen nahe, dass dieser Mechanismus auch für Fettgewebemakrophagen gültig ist, dass aber gleichzeitig auch die Zytokine Interleukin 13 (IL-13) und Interleukin 6 (IL-6) die Proliferation von Fettgewebemakrophagen

beeinflussen (Braune et al. 2017). Interessanterweise scheint IL-6, welches gemeinhin als proinflammatorisches Zytokin gilt, im entzündeten Fettgewebe adipöser Individuen über die Regulation des IL-4-Rezeptors eher eine antiinflammatorische Rolle zu spielen (Mauer et al. 2014).

## 1.8 Fettgewebelymphozyten

Neben den Makrophagen scheint auch das adaptive Immunsystem an der Ausprägung der adipositasinduzierten Fettgewebeentzündung und Insulinresistenz beteiligt zu sein (Osborn et Olefsky 2012), wobei Art und Ausmaß dieser Beteiligung kontrovers diskutiert werden (Tabelle 1 und 2). So konnte zum Beispiel gezeigt werden, dass Mäuse, denen das *recombination activating gene 1* (RAG-1) fehlt und damit die Fähigkeit, jegliche Art von Lymphozyten zu bilden, im Vergleich zu Kontrolltieren unter einer hochkalorischen Diät eine größere Fettmasse und eine Verschlechterung der Stoffwechselparameter aufweisen (Winer et al. 2009). Demgegenüber führten die Depletionen von CD3-positiven T-Zellen, CD8-positiven T-Zellen und CD20-positiven B-Zellen allesamt zu einer Abschwächung der Fettgewebeinflammation in Form einer relativen Reduktion der proinflammatorischen M1-Makrophagen und einer Verbesserung der metabolischen Parameter (Winer et al. 2009; Winer et al. 2011; Nishimura et al. 2009). Diese Studien führten zu der Vorstellung, dass die Lymphozyten in ihrer Gesamtheit zunächst eher eine protektive Rolle besitzen, dass diese protektive Rolle allerdings im Verlauf der hochkalorischen Fütterung verloren geht und daher die Depletion dieser Zellen in adipösen Individuen die gestörte Fettgewebeintegrität und den gestörten Glukosestoffwechsel teilweise wiederherstellt. Im Gegensatz dazu konnte eine andere Studie an Mäusen ohne RAG-1 im Vergleich zu Wildtyp-Mäusen keine Veränderung der Stoffwechselparameter feststellen (Liu et al. 2015). Diese negativen Ergebnisse konnten an Mäusen ohne das *recombination activating gene 2* (RAG-2), die einen ähnlichen Mangel an Lymphozyten aufweisen wie RAG-1 *knockout*- (KO) Mäuse, reproduziert werden (Duffaut et al. 2009). Schwer vereinbar mit der Vorstellung von metabolisch protektiven Lymphozyten, ist auch die Feststellung, dass Mäuse ohne *T cell receptor beta chain* (TCRb), denen die Mehrheit aller T-Zellen fehlt, zwar einen Anstieg der Fettmasse zeigen, dies jedoch einhergeht mit einer Verringerung der M1-Makrophagen und einer Verbesserung der Stoffwechselparameter (Khan et al. 2014). Weitere Studien an RAG-1 KO-Mäusen haben außerdem nahegelegt, dass Lymphozyten weder für die Proliferation, noch für die Polarisierung von Fettgewebemakrophagen zwingend erforderlich sind (Muir et al. 2018; Zamarron et al. 2017).

### 1.8.1 CD4-positive Fettgewebelymphozyten

Nach einem in den frühen achtziger Jahren etablierten Modell, werden alle reifen, peripheren T-Lymphozyten anhand der Expression von CD3 charakterisiert und diese CD3-positiven T-Lymphozyten lassen sich weiter anhand der Expression von CD4 und CD8 auf ihrer Zelloberfläche differenzieren (Reinherz 2014; Reinherz et al. 1979, 1980). Im Fettgewebe postulieren mehrere Studien eine relativ frühe Expansion der CD3-positiven T-Zellen unter einer hochkalorischen Diät (Deng et al. 2013; Kintscher et al. 2008). Nishimura et al. gehen nun allerdings davon aus, dass innerhalb der CD3-positiven Population, die CD8-positive Fraktion unter einer hochkalorischen Diät relativ zunimmt, wohingegen die CD4-Fraktion relativ abnimmt (Nishimura et al. 2009). Andere beschreiben jedoch eine relativ stabile CD4-Fraktion unter hochkalorischer Diät (Strissel et al. 2010) und wieder andere zeigen einen relativen Anstieg der CD4-positiven T-Zellen (Shirakawa et al. 2016). Auch die Beschreibungen der phänotypischen Veränderungen innerhalb der CD4-positiven Population sind relativ heterogen. Ein großer Konsens besteht allerdings im Hinblick auf die Population der CD4-positiven regulatorischen T-Zellen (Tregs). Diese exprimieren den Transkriptionsfaktor *forkhead box protein 3* (FOXP3) und spielen eine wichtige Rolle in der Kontrolle der gewebespezifischen Homöostase und der Immuntoleranz (Becker, Levings et Daniel 2017). Die Population der fettgeweberesidenten Tregs nimmt normalerweise mit der Zeit zu und kann abhängig vom Alter bis zu 50% der CD4-positiven Zellen im Fettgewebe ausmachen (Feuerer et al. 2009). Eine hochkalorische Fütterung führt allerdings in Mäusen zu einer relativen Abnahme der Tregs (Feuerer et al. 2009; Nishimura et al. 2009; Winer et al. 2009; Bapat et al. 2015; Cipolletta et al. 2015). Interessanterweise ist diese Abnahme der Tregs im Verlauf der adipositasinduzierten Inflammation lediglich im viszeralen, nicht aber im subkutanen Fettgewebe zu beobachten (Feuerer et al. 2009). Der Vollständigkeit halber soll jedoch nicht unerwähnt bleiben, dass Cho et al. diesen diätabhängigen *shift* der Fettgewebe-Tregs nicht reproduzieren konnten (Cho et al. 2014). *Suppression of tumorigenicity 2* (ST2) ist ein weiterer Marker, dessen Expression im murinen Fettgewebe eng mit Tregs assoziiert ist und ähnlich wie die Expression von FOXP3 scheint auch die Expression von ST2 unter dem Einfluss einer hochkalorischen Diät herunterreguliert zu werden (Han et al. 2015a; Vasanthakumar et al. 2015). Sowohl FOXP3 als auch ST2 werden im Rahmen diätinduzierten Fettgewebeinflammation eher antiinflammatorische Eigenschaften zugeschrieben und mehrere Studien haben eine modulierende Funktion auf den Glukosemetabolismus und die Fettgewebeinflammation nahegelegt. In diesem Sinne verwundert es nicht, dass mehrere Studien zeigen konnten, dass die Expansion der Treg-Population im Fettgewebe Parameter der Fettgewebeinflammation und des Stoffwechsels positiv beeinflusst (Eller et al. 2011; Han et al. 2015a; Vasanthakumar et al. 2015). Im Gegensatz dazu haben Untersuchungen an Mäusen mit gentechnisch induziertem Mangel an regulatorischen T-Zellen unter hochkalorischer Diät im Vergleich zum Wildtyp keine Beeinflussung des

Glukosestoffwechsels feststellen können (Bapat et al. 2015; Cipolletta et al. 2012; Deng et al. 2017)

Die Produktion des Zytokins Interferon-γ (IFNg) ist charakteristisch für eine weitere CD4-positive Population, welche mittelbar mit Veränderungen des Glukosestoffwechsels in Zusammenhang gebracht wird. Diese Vorstellung beruht zum Teil auf der Beobachtung, dass Mäuse, denen die Fähigkeit fehlt, IFNg zu bilden, unter hochkalorischer Diät einen insulinsensitiveren Phänotyp und einen geringeren Anstieg proinflammatorischer Makrophagen zeigen als die Kontrolltiere (O'Rourke et al. 2012). Die Vorstellung metabolisch schädlicher, IFNg-produzierender Zellen ist daher vordergründig vereinbar mit einer relativen Zunahme IFNg-produzierenden CD4-Zellen im Verlauf einer hochkalorischen Diät (Winer et al. 2009). Eine andere Studie beobachtete allerdings eine Abnahme der IFNg-positiven Zellen (Zamarron et al. 2017).

Die Rolle der CD4-positiven Lymphozyten im Rahmen der Fettgewebeinflammation ist daher aus diesen Vorbefunden schwer zu interpretieren. Hinweise liefert die Arbeit von Winer et al., in der die Autoren beschreiben, dass die Verschlechterung der Glukosetoleranz, welche sie in RAG-1-Mäusen festgestellt haben, durch den Transfer von sowohl FOXP3-positiven, als auch FOXP3-negativen CD4-Zellen aus schlanken Tieren, teilweise wiederhergestellt werden konnte (Winer et al. 2009). Diese Ergebnisse lassen vermuten, dass CD4-Zellen unbeeinträchtigt von pathologischen Einflussfaktoren zunächst einen metabolisch protektiven Phänotyp besitzen. Schwer vereinbar mit diesem Postulat sind Untersuchungen von Mäusen mit einem Myeloidzell-spezifischen Mangel an *major histocompatibility complex II* (MHC II), bei denen begleitend eine starke Depletion von CD4-positiven T-Zellen beobachtet werden konnte. Dieses Modell führte in einer Studie zu einem insulinsensitiveren Phänotyp und einer Reduktion proinflammatorischer Makrophagen (Cho et al. 2014). Eine andere Studie konnte diesen Phänotyp jedoch nicht reproduzieren (Blaszczak et al. 2019). Insgesamt kann man festhalten, dass unsere Vorstellung über die Rolle von CD4-positiven T-Zellen im Rahmen der adipositasinduzierten Fettgewebeentzündung vor allem aus genetischen *knockout*-Modellen extrapoliert wurde, diese Vorstellung jedoch noch nicht in einem *In-vivo*-Depletionsmodell validiert werden konnte.

| Untersuchte Population | Fraktion unter HFD | Publikation |
|---|---|---|
| $CD4^+$ (an $CD3^+/CD45^+$) | ↓ | Nishimura et al. 2009 |
| | ↑ | Shirakawa et al. 2016 |
| | ↓ | Winer et al. 2009 |
| | → | Brinker et al. 2021 |
| $CD4^+/FOXP3^+$ (an $CD4^+$) | ↓ | Feuerer et al. 2009 |
| | ↓ | Nishimura et al. 2009 |
| | ↓ | Winer et al. 2009 |
| | ↓ | Bapat et al. 2015 |
| | ↓ | Cipolletta et al. 2012 |
| | → | Cho et al. 2014 |
| | ↓ | Brinker et al. 2021 |
| $CD4^+/IFNg^+$ (an $CD4^+$) | ↑ | Winer et al. 2009 |
| | ↓ | Zamarron et al. 2017 |
| | → | Brinker et al. 2021 |

Tabelle 1: Veränderung der Fraktionen von CD4-positiven Populationen im murinen Fettgewebe unter hochkalorischer Diät (HFD) im Vergleich zur Normaldiät.

| Modulation | MΦ-akkumulation nach HFD | M1/M2-Ratio nach HFD | Glykämische Stoffwechsellage nach HFD | Publikation |
|---|---|---|---|---|
| RAG-1-KO | KA | KA | ↓ | Winer et al. 2009 |
| | KA | KA | → | Liu et al. 2015 |
| TCRb-KO | KA | ↓ | ↑ | Khan et al. 2014 |
| Myeloidzell-MHCII-KO | ↓ | ↓ | ↑ | Cho et al. 2014 |
| | ↓ | ↓ | → | Blaszczak et al. 2019 |
| Treg-Expansion (IL-33 i.p.) | ↓ | KA | ↑ | Han et al. 2015 |
| | ↓ | KA | ↑ | Vasanthakumar et al. 2015 |
| Treg-Depletion (PPARG-KO) | → | KA | → | Bapat et al. 2015 |
| | KA | KA | → | Cipolletta et al. 2012 |
| IFNg-KO | KA | ↓ | ↑ | O'Rourke et al. 2012 |
| CD4-Depletion | → | → | ↑ | Brinker et al. 2021 |

Tabelle 2: Veränderung der Makrophagenakkumulation, Makrophagenaktivierung und der metabolischen Parameter im Mausmodell der diätinduzierten Adipositas unter dem Einfluss verschiedener *in-vivo*-Modulationen der CD4-positiven Populationen. MΦ Makrophagen, KA Keine Angaben, ↑ Verbesserung/mehr, → Kein Einfluss, ↓ Verschlechterung/weniger.

# 2 Fragestellung

Aus diesen Vorbefunden ergaben sich für uns folgende Fragestellungen:

1. Wie verändert sich die Größe und Zusammensetzung der Lymphozytenpopulation im Fettgewebe mit der Dauer einer hochkalorischen Diät im Mausmodell?
2. Welchen Einfluss hat die Depletion CD4-positiver Zellen auf den Aktivitätszustand und die Proliferationsrate der Fettgewebemakrophagen?
3. Beeinflusst die Dauer der Lymphozytendepletion den Nachweis eines Effektes auf den Aktivitätszustand und die Proliferationsrate der Fettgewebemakrophagen?
4. Welchen Einfluss hat die Depletion CD4-positiver Zellen auf die Glukosehomöostase?

Um diese Fragen beantworten zu können, konzipierten wir ein *In-vivo*-Depletionsprotokoll für CD4-positive Zellen in Übereinstimmung mit bereits etablierten Depletionsprotokollen. Methodisch kamen im Verlauf der Arbeit vor allem die Durchflusszytometrie, die Immunfluoreszenzmikroskopie und die Polymerase-Kettenreaktion zum Einsatz. Darüber hinaus führten wir Insulinsensitivitäts- und Glukosetoleranztestungen durch.

# 3 Literaturverzeichnis der Einführung


Amano, Shinya U.; Cohen, Jessica L.; Vangala, Pranitha; Tencerova, Michaela; Nicoloro, Sarah M.; Yawe, Joseph C. et al. (2014) Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. In : Cell metabolism, vol. 19, n° 1, p. 162–171.

Bapat, Sagar P.; Myoung Suh, Jae; Fang, Sungsoon; Liu, Sihao; Zhang, Yang; Cheng, Albert et al. (2015) Depletion of fat-resident Treg cells prevents age-associated insulin resistance. In : Nature, vol. 528, n° 7580, p. 137–141.

Becker, Maike; Levings, Megan K.; Daniel, Carolin (2017) Adipose-tissue regulatory T cells. Critical players in adipose-immune crosstalk. In : European journal of immunology, vol. 47, n° 11, p. 1867–1874.

Bhaskaran, Krishnan; Douglas, Ian; Forbes, Harriet; dos-Santos-Silva, Isabel; Leon, David A.; Smeeth, Liam (2014) Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5·24 million UK adults. In : The Lancet, vol. 384, n° 9945, p. 755–765.

Billon, Nathalie; Monteiro, Miguel Caetano; Dani, Christian (2008) Developmental origin of adipocytes: new insights into a pending question. In : Biology of the Cell, vol. 100, n° 10, p. 563–575.

Birsoy, Kivanç; Festuccia, William T.; Laplante, Mathieu (2013) A comparative perspective on lipid storage in animals. In : Journal of Cell Science, vol. 126, n° Pt 7, p. 1541–1552.

Blaszczak, Alecia M.; Wright, Valerie P.; Anandani, Kajol; Liu, Joey; Jalilvand, Anahita; Bergin, Stephen et al. (2019) Loss of Antigen Presentation in Adipose Tissue Macrophages or in Adipocytes, but Not Both, Improves Glucose Metabolism. In : Journal of immunology (Baltimore, Md. : 1950), vol. 202, n° 8, p. 2451–2459.

Bourlier, V.; Zakaroff-Girard, A.; Miranville, A.; Barros, S. de; Maumus, M.; Sengenes, C. et al. (2008) Remodeling phenotype of human subcutaneous adipose tissue macrophages. In : Circulation, vol. 117, n° 6, p. 806–815.

Braune, Julia; Weyer, Ulrike; Hobusch, Constance; Mauer, Jan; Brüning, Jens C.; Bechmann, Ingo; Gericke, Martin (2017) IL-6 Regulates M2 Polarization and Local Proliferation of Adipose Tissue Macrophages in Obesity. In : Journal of immunology (Baltimore, Md. : 1950), vol. 198, n° 7, p. 2927–2934.

Brestoff, Jonathan R.; Kim, Brian S.; Saenz, Steven A.; Stine, Rachel R.; Monticelli, Laurel A.; Sonnenberg, Gregory F. et al. (2015) Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. In : Nature, vol. 519, n° 7542, p. 242–246.

Brestoff, Jonathan R.; Wilen, Craig B.; Moley, John R.; Li, Yongjia; Zou, Wei; Malvin, Nicole P. et al. (2021) Intercellular Mitochondria Transfer to Macrophages Regulates White Adipose Tissue Homeostasis and Is Impaired in Obesity. In : Cell metabolism, vol. 33, n° 2, 270-282.e8.

Brinker, Georg; Froeba, Janine; Arndt, Lilli; Braune, Julia; Hobusch, Constance; Lindhorst, Andreas et al. (2021) CD4+ T cells regulate glucose homeostasis independent of adipose tissue dysfunction in mice. In : European journal of immunology. (Im Druck)

Carey, V. J.; Walters, E. E.; Colditz, G. A.; Solomon, C. G.; Willett, W. C.; Rosner, B. A. et al. (1997) Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. In : American Journal of Epidemiology, vol. 145, n° 7, p. 614–619.

Cawthorn, William P.; Scheller, Erica L.; MacDougald, Ormond A. (2012) Adipose tissue stem cells meet preadipocyte commitment: going back to the future. In : Journal of lipid research, vol. 53, n° 2, p. 227–246.

Chawla, Ajay; Nguyen, Khoa D.; Goh, Y. P. Sharon (2011) Macrophage-mediated inflammation in metabolic disease. In : Nature Reviews Immunology, vol. 11, n° 11, p. 738–749.

Cho, Kae Won; Morris, David L.; DelProposto, Jennifer L.; Geletka, Lynn; Zamarron, Brian; Martinez-Santibanez, Gabriel et al. (2014) An MHC II-dependent activation loop between adipose tissue macrophages and CD4+ T cells controls obesity-induced inflammation. In : Cell reports, vol. 9, n° 2, p. 605–617.

Cinti, Saverio; Mitchell, Grant; Barbatelli, Giorgio; Murano, Incoronata; Ceresi, Enzo; Faloia, Emanuela et al. (2005) Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. In : Journal of lipid research, vol. 46, n° 11, p. 2347–2355.

Cipolletta, Daniela; Cohen, Paul; Spiegelman, Bruce M.; Benoist, Christophe; Mathis, Diane (2015) Appearance and disappearance of the mRNA signature characteristic of Treg cells in visceral adipose tissue. Age, diet, and PPARγ effects. In : Proceedings of the National Academy of Sciences of the United States of America, vol. 112, n° 2, p. 482–487.

Cipolletta, Daniela; Feuerer, Markus; Li, Amy; Kamei, Nozomu; Lee, Jongsoon; Shoelson, Steven E. et al. (2012) PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. In : Nature, vol. 486, n° 7404, p. 549–553.

Cohen, Paul; Spiegelman, Bruce M. (2015) Brown and Beige Fat. Molecular Parts of a Thermogenic Machine. In : Diabetes, vol. 64, n° 7, p. 2346–2351.

Cypess, Aaron M.; Kahn, C. Ronald (2010) Brown fat as a therapy for obesity and diabetes. In : Current opinion in endocrinology, diabetes, and obesity, vol. 17, n° 2, p. 143–149.

Dasu, Mohan R.; Devaraj, Sridevi; Park, Samuel; Jialal, Ishwarlal (2010) Increased toll-like receptor (TLR) activation and TLR ligands in recently diagnosed type 2 diabetic subjects. In : Diabetes care, vol. 33, n° 4, p. 861–868.

Davies, Luke C.; Jenkins, Stephen J.; Allen, Judith E.; Taylor, Philip R. (2013) Tissue-resident macrophages. In : Nature immunology, vol. 14, n° 10, p. 986–995.

Deng, Tuo; Liu, Joey; Deng, Yanru; Minze, Laurie; Xiao, Xiang; Wright, Valerie et al. (2017) Adipocyte adaptive immunity mediates diet-induced adipose inflammation and insulin resistance by decreasing adipose Treg cells. In : Nature Communications, vol. 8, n° 1, p. 1–11.

Deng, Tuo; Lyon, Christopher J.; Minze, Laurie J.; Lin, Jianxin; Zou, Jia; Liu, Joey Z. et al. (2013) Class II major histocompatibility complex plays an essential role in obesity-induced adipose inflammation. In : Cell metabolism, vol. 17, n° 3, p. 411–422.

Di Angelantonio, Emanuele; Bhupathiraju, Shilpa N.; Wormser, David; Gao, Pei; Kaptoge, Stephen; Gonzalez, Amy Berrington de et al. (2016) Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. In : The Lancet, vol. 388, n° 10046, p. 776–786.

Duffaut, Carine; Galitzky, Jean; Lafontan, Max; Bouloumié, Anne (2009) Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. In : Biochemical and biophysical research communications, vol. 384, n° 4, p. 482–485.

Eller, Kathrin; Kirsch, Alexander; Wolf, Anna M.; Sopper, Sieghart; Tagwerker, Andrea; Stanzl, Ursula et al. (2011) Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. In : Diabetes, vol. 60, n° 11, p. 2954–2962.

Feuerer, Markus; Herrero, Laura; Cipolletta, Daniela; Naaz, Afia; Wong, Jamie; Nayer, Ali et al. (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. In : Nature medicine, vol. 15, n° 8, p. 930–939.

Fox, Caroline S.; Massaro, Joseph M.; Hoffmann, Udo; Pou, Karla M.; Maurovich-Horvat, Pal; Liu, Chun-Yu et al. (2007) Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. In : Circulation, vol. 116, n° 1, p. 39–48.

Frontini, Andrea; Cinti, Saverio (2010) Distribution and development of brown adipocytes in the murine and human adipose organ. In : Cell metabolism, vol. 11, n° 4, p. 253–256.

Fukuhara, Atsunori; Matsuda, Morihiro; Nishizawa, Masako; Segawa, Katsumori; Tanaka, Masaki; Kishimoto, Kae et al. (2005) Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. In : Science (New York, N.Y.), vol. 307, n° 5708, p. 426–430.

Gastaldelli, Amalia; Miyazaki, Yoshinori; Pettiti, Maura; Matsuda, Masafumi; Mahankali, Srihanth; Santini, Eleonora et al. (2002) Metabolic effects of visceral fat accumulation in type 2 diabetes. In : The Journal of clinical endocrinology and metabolism, vol. 87, n° 11, p. 5098–5103.

Glass, Christopher K.; Olefsky, Jerrold M. (2012) Inflammation and lipid signaling in the etiology of insulin resistance. In : Cell metabolism, vol. 15, n° 5, p. 635–645.

Gordon, Siamon (2003) Alternative activation of macrophages. In : Nature reviews. Immunology, vol. 3, n° 1, p. 23–35.

Haase, Julia; Weyer, Ulrike; Immig, Kerstin; Klöting, Nora; Blüher, Matthias; Eilers, Jens et al. (2014) Local proliferation of macrophages in adipose tissue during obesity-induced inflammation. In : Diabetologia, vol. 57, n° 3, p. 562–571.

Han, Jonathan M.; Wu, Dan; Denroche, Heather C.; Yao, Yu; Verchere, C. Bruce; Levings, Megan K. (2015) IL-33 Reverses an Obesity-Induced Deficit in Visceral Adipose Tissue ST2+ T Regulatory Cells and Ameliorates Adipose Tissue Inflammation and Insulin Resistance. In : Journal of immunology (Baltimore, Md. : 1950), vol. 194, n° 10, p. 4777–4783.

Hashimoto, Daigo; Chow, Andrew; Noizat, Clara; Teo, Pearline; Beasley, Mary Beth; Leboeuf, Marylene et al. (2013) Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. In : Immunity, vol. 38, n° 4, p. 792–804.

Hassnain Waqas, Syed F.; Noble, Anna; Hoang, Anh C.; Ampem, Grace; Popp, Manuela; Strauß, Sarah et al. (2017) Adipose tissue macrophages develop from bone marrow-independent progenitors in Xenopus laevis and mouse. In : Journal of leukocyte biology, vol. 102, n° 3, p. 845–855.

Heinonen, Sini; Buzkova, Jana; Muniandy, Maheswary; Kaksonen, Risto; Ollikainen, Miina; Ismail, Khadeeja et al. (2015) Impaired Mitochondrial Biogenesis in Adipose Tissue in Acquired Obesity. In : Diabetes, vol. 64, n° 9, p. 3135–3145.

Jenkins, Stephen J.; Ruckerl, Dominik; Cook, Peter C.; Jones, Lucy H.; Finkelman, Fred D.; van Rooijen, Nico et al. (2011) Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. In : Science (New York, N.Y.), vol. 332, n° 6035, p. 1284–1288.

Jiang, Changtao; Qu, Aijuan; Matsubara, Tsutomu; Chanturiya, Tatyana; Jou, William; Gavrilova, Oksana et al. (2011) Disruption of hypoxia-inducible factor 1 in adipocytes improves insulin sensitivity and decreases adiposity in high-fat diet-fed mice. In : Diabetes, vol. 60, n° 10, p. 2484–2495.

Kanda, Hajime; Tateya, Sanshiro; Tamori, Yoshikazu; Kotani, Ko; Hiasa, Ken-ichi; Kitazawa, Riko et al. (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. In : The Journal of Clinical Investigation, vol. 116, n° 6, p. 1494–1505.

Khan, Ilvira M.; Dai Perrard, Xiao-Yuan; Perrard, Jerry L.; Mansoori, Amir; Smith, C. Wayne; Wu, Huaizhu; Ballantyne, Christie M. (2014) Attenuated adipose tissue and skeletal muscle inflammation in obese mice with combined CD4+ and CD8+ T cell deficiency. In : Atherosclerosis, vol. 233, n° 2, p. 419–428.

Khan, Tayeba; Muise, Eric S.; Iyengar, Puneeth; Wang, Zhao V.; Chandalia, Manisha; Abate, Nicola et al. (2009) Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. In : Molecular and cellular biology, vol. 29, n° 6, p. 1575–1591.

Kiefer, Florian W.; Zeyda, Maximilian; Gollinger, Karina; Pfau, Birgit; Neuhofer, Angelika; Weichhart, Thomas et al. (2010) Neutralization of osteopontin inhibits obesity-induced inflammation and insulin resistance. In : Diabetes, vol. 59, n° 4, p. 935–946.

Kintscher, Ulrich; Hartge, Martin; Hess, Katharina; Foryst-Ludwig, Anna; Clemenz, Markus; Wabitsch, Martin et al. (2008) T-lymphocyte infiltration in visceral adipose tissue. A primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. In : Arteriosclerosis, thrombosis, and vascular biology, vol. 28, n° 7, p. 1304–1310.

Kivimäki, Mika; Kuosma, Eeva; Ferrie, Jane E.; Luukkonen, Ritva; Nyberg, Solja T.; Alfredsson, Lars et al. (2017) Overweight, obesity, and risk of cardiometabolic multimorbidity: pooled analysis of individual-level data for 120 813 adults from 16 cohort studies from the USA and Europe. In : The Lancet Public Health, vol. 2, n° 6, e277-e285.

Könner, A. Christine; Brüning, Jens C. (2011) Toll-like receptors: linking inflammation to metabolism. In : Trends in Endocrinology & Metabolism, vol. 22, n° 1, p. 16–23.

Kosteli, Aliki; Sugaru, Eiji; Haemmerle, Guenter; Martin, Jayne F.; Lei, Jason; Zechner, Rudolf; Ferrante, Anthony W. (2010) Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. In : The Journal of Clinical Investigation, vol. 120, n° 10, p. 3466–3479.

Kruglikov, Ilja L.; Scherer, Philipp E. (2016) Dermal Adipocytes: From Irrelevance to Metabolic Targets? In : Trends in Endocrinology & Metabolism, vol. 27, n° 1, p. 1–10.

Kurokawa, Jun; Nagano, Hiromichi; Ohara, Osamu; Kubota, Naoto; Kadowaki, Takashi; Arai, Satoko; Miyazaki, Toru (2011) Apoptosis inhibitor of macrophage (AIM) is required for obesity-associated recruitment of inflammatory macrophages into adipose tissue. In : Proceedings of the National Academy of Sciences, vol. 108, n° 29, p. 12072–12077.

Kusminski, Christine M.; Bickel, Perry E.; Scherer, Philipp E. (2016) Targeting adipose tissue in the treatment of obesity-associated diabetes. In : Nature Reviews Drug Discovery, vol. 15, n° 9, p. 639–660.

Kwok, Kelvin H. M.; Lam, Karen S. L.; Xu, Aimin (2016) Heterogeneity of white adipose tissue: molecular basis and clinical implications. In : Experimental & Molecular Medicine, vol. 48, n° 3, e215.

Lee, Kevin Y.; Gesta, Stephane; Boucher, Jeremie; Wang, Xiaohui L.; Kahn, C. Ronald (2011) The differential role of Hif1β/Arnt and the hypoxic response in adipose function, fibrosis, and inflammation. In : Cell metabolism, vol. 14, n° 4, p. 491–503.

Lee, Mi-Jeong; Wu, Yuanyuan; Fried, Susan K. (2013) Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. In : Molecular Aspects of Medicine, vol. 34, n° 1, p. 1–11.

Lee, Yun Sok; Kim, Jung-whan; Osborne, Olivia; Oh, Da Young; Sasik, Roman; Schenk, Simon et al. (2014) Increased adipocyte O2 consumption triggers HIF-1α, causing inflammation and insulin resistance in obesity. In : Cell, vol. 157, n° 6, p. 1339–1352.

Lee, Yun Sok; Wollam, Joshua; Olefsky, Jerrold M. (2018a) An Integrated View of Immunometabolism. In : Cell, vol. 172, n° 1-2, p. 22–40.

Lee, Yun Sok; Wollam, Joshua; Olefsky, Jerrold M. (2018b) An Integrated View of Immunometabolism. In : Cell, vol. 172, n° 1-2, p. 22–40.

Li, Qinkai; Hata, Akiko; Kosugi, Chisato; Kataoka, Nananko; Funaki, Makoto (2010) The density of extracellular matrix proteins regulates inflammation and insulin signaling in adipocytes. In : FEBS letters, vol. 584, n° 19, p. 4145–4150.

Liu, Jian; Divoux, Adeline; Sun, Jiusong; Zhang, Jie; Clément, Karine; Glickman, Jonathan N. et al. (2009) Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. In : Nature medicine, vol. 15, n° 8, p. 940–945.

Liu, X.; Huh, J. Y.; Gong, H.; Chamberland, J. P.; Brinkoetter, M. T.; Hamnvik, O-P R.; Mantzoros, C. S. (2015) Lack of mature lymphocytes results in obese but metabolically healthy mice when fed a high-fat diet. In : International journal of obesity (2005), vol. 39, n° 10, p. 1548–1557.

Lumeng, Carey N.; Bodzin, Jennifer L.; Saltiel, Alan R. (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. In : The Journal of clinical investigation, vol. 117, n° 1, p. 175–184.

Lumeng, Carey N.; DelProposto, Jennifer B.; Westcott, Daniel J.; Saltiel, Alan R. (2008) Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. In : Diabetes, vol. 57, n° 12, p. 3239–3246.

Lynch, Lydia; Nowak, Michael; Varghese, Bindu; Clark, Justice; Hogan, Andrew E.; Toxavidis, Vasillis et al. (2012) Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. In : Immunity, vol. 37, n° 3, p. 574–587.

Mantovani, Alberto; Sica, Antonio; Sozzani, Silvano; Allavena, Paola; Vecchi, Annunciata; Locati, Massimo (2004) The chemokine system in diverse forms of macrophage activation and polarization. In : TRENDS IN IMMUNOLOGY, vol. 25, n° 12, p. 677–686.

Martinez, Fernando O.; Gordon, Siamon (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. In : F1000prime reports, vol. 6, p. 13.

Mauer, Jan; Chaurasia, Bhagirath; Goldau, Julia; Vogt, Merly C.; Ruud, Johan; Nguyen, Khoa D. et al. (2014) Signaling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. In : Nature Immunology, vol. 15, n° 5, p. 423–430.

McLaughlin, Tracey; Lamendola, Cindy; Liu, Alice; Abbasi, Fahim (2011) Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. In : The Journal of clinical endocrinology and metabolism, vol. 96, n° 11, E1756-60.

Miyazaki, Yoshinori; DeFronzo, Ralph A. (2009) Visceral fat dominant distribution in male type 2 diabetic patients is closely related to hepatic insulin resistance, irrespective of body type. In : Cardiovascular Diabetology, vol. 8, n° 1, p. 44.

Muir, Lindsey A.; Kiridena, Samadhi; Griffin, Cameron; DelProposto, Jennifer B.; Geletka, Lynn; Martinez-Santibañez, Gabriel et al. (2018) Frontline Science: Rapid adipose tissue expansion triggers unique proliferation and lipid accumulation profiles in adipose tissue macrophages. In : Journal of leukocyte biology, vol. 103, n° 4, p. 615–628.

Murano, I.; Barbatelli, G.; Parisani, V.; Latini, C.; Muzzonigro, G.; Castellucci, M.; Cinti, S. (2008) Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. In : Journal of lipid research, vol. 49, n° 7, p. 1562–1568.

Nakatani, Yoshihisa; Kaneto, Hideaki; Kawamori, Dan; Yoshiuchi, Kazutomi; Hatazaki, Masahiro; Matsuoka, Taka-aki et al. (2005) Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. In : Journal of Biological Chemistry, vol. 280, n° 1, p. 847–851.

Ng, Marie; Fleming, Tom; Robinson, Margaret; Thomson, Blake; Graetz, Nicholas; Margono, Christopher et al. (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. In : The Lancet, vol. 384, n° 9945, p. 766–781.

Nguyen, M. T. Audrey; Favelyukis, Svetlana; Nguyen, Anh-Khoi; Reichart, Donna; Scott, Peter A.; Jenn, Alan et al. (2007) A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. In : Journal of Biological Chemistry, vol. 282, n° 48, p. 35279–35292.

Nishimura, Satoshi; Manabe, Ichiro; Nagasaki, Mika; Eto, Koji; Yamashita, Hiroshi; Ohsugi, Mitsuru et al. (2009) CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. In : Nature medicine, vol. 15, n° 8, p. 914–920.

Nomiyama, Takashi; Perez-Tilve, Diego; Ogawa, Daisuke; Gizard, Florence; Zhao, Yue; Heywood, Elizabeth B. et al. (2007) Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. In : The Journal of clinical investigation, vol. 117, n° 10, p. 2877–2888.

O'Rourke, R. W.; Metcalf, M. D.; White, A. E.; Madala, A.; Winters, B. R.; Maizlin, I. I. et al. (2009) Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-gamma in inflammation in human adipose tissue. In : International journal of obesity (2005), vol. 33, n° 9, p. 978–990.

O'Rourke, Robert W.; White, Ashley E.; Metcalf, Monja D.; Winters, Brian R.; Diggs, Brian S.; Zhu, Xinxia; Marks, Daniel L. (2012) Systemic inflammation and insulin sensitivity in obese IFN-γ knockout mice. In : Metabolism: clinical and experimental, vol. 61, n° 8, p. 1152–1161.

Osborn, Olivia; Olefsky, Jerrold M. (2012) The cellular and signaling networks linking the immune system and metabolism in disease. In : Nature medicine, vol. 18, n° 3, p. 363–374.

Ozcan, Umut; Cao, Qiong; Yilmaz, Erkan; Lee, Ann-Hwee; Iwakoshi, Neal N.; Ozdelen, Esra et al. (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. In : Science (New York, N.Y.), vol. 306, n° 5695, p. 457–461.

Ozcan, Umut; Yilmaz, Erkan; Ozcan, Lale; Furuhashi, Masato; Vaillancourt, Eric; Smith, Ross O. et al. (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a

mouse model of type 2 diabetes. In : Science (New York, N.Y.), vol. 313, n° 5790, p. 1137–1140.

Patsouris, David; Li, Ping-Ping; Thapar, Divya; Chapman, Justin; Olefsky, Jerrold M.; Neels, Jaap G. (2008) Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. In : Cell metabolism, vol. 8, n° 4, p. 301–309.

Qatanani, Mohammed; Tan, Yejun; Dobrin, Radu; Greenawalt, Danielle M.; Hu, Guanghui; Zhao, Wenqing et al. (2013) Inverse regulation of inflammation and mitochondrial function in adipose tissue defines extreme insulin sensitivity in morbidly obese patients. In : Diabetes, vol. 62, n° 3, p. 855–863.

Reilly, Shannon M.; Saltiel, Alan R. (2017) Adapting to obesity with adipose tissue inflammation. In : Nature Reviews Endocrinology, vol. 13, n° 11, p. 633–643.

Reinherz, E. L.; Kung, P. C.; Goldstein, G.; Schlossman, S. F. (1979) Separation of functional subsets of human T cells by a monoclonal antibody. In : Proceedings of the National Academy of Sciences of the United States of America, vol. 76, n° 8, p. 4061–4065.

Reinherz, E. L.; Kung, P. C.; Goldstein, G.; Schlossman, S. F. (1980) A monoclonal antibody reactive with the human cytotoxic/suppressor T cell subset previously defined by a heteroantiserum termed TH2. In : The Journal of Immunology, vol. 124, n° 3, p. 1301–1307.

Reinherz, Ellis L. (2014) Revisiting the Discovery of the αβ TCR Complex and Its Co-Receptors. In : Frontiers in Immunology, vol. 5, p. 583.

Rosen, Evan D.; Spiegelman, Bruce M. (2014) What we talk about when we talk about fat. In : Cell, vol. 156, n° 1-2, p. 20–44.

Russo, Lucia; Lumeng, Carey N. (2018) Properties and functions of adipose tissue macrophages in obesity. In : Immunology, vol. 155, n° 4, p. 407–417.

Rutkowski, Joseph M.; Stern, Jennifer H.; Scherer, Philipp E. (2015) The cell biology of fat expansion. In : Journal of Cell Biology, vol. 208, n° 5, p. 501–512.

Saltiel, A. R.; Kahn, C. R. (2001) Insulin signalling and the regulation of glucose and lipid metabolism. In : Nature, vol. 414, n° 6865, p. 799–806.

Scherer, P. E.; Williams, S.; Fogliano, M.; Baldini, G.; Lodish, H. F. (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. In : The Journal of biological chemistry, vol. 270, n° 45, p. 26746–26749.

Schulz, Christian; Gomez Perdiguero, Elisa; Chorro, Laurent; Szabo-Rogers, Heather; Cagnard, Nicolas; Kierdorf, Katrin et al. (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. In : Science (New York, N.Y.), vol. 336, n° 6077, p. 86–90.

Seale, Patrick; Bjork, Bryan; Yang, Wenli; Kajimura, Shingo; Chin, Sherry; Kuang, Shihuan et al. (2008) PRDM16 controls a brown fat/skeletal muscle switch. In : Nature, vol. 454, n° 7207, p. 961–967.

Shirakawa, Kohsuke; Yan, Xiaoxiang; Shinmura, Ken; Endo, Jin; Kataoka, Masaharu; Katsumata, Yoshinori et al. (2016) Obesity accelerates T cell senescence in murine visceral adipose tissue. In : The Journal of clinical investigation, vol. 126, n° 12, p. 4626–4639.

Shoelson, Steven E.; Lee, Jongsoon; Goldfine, Allison B. (2006) Inflammation and insulin resistance. In : The Journal of Clinical Investigation, vol. 116, n° 7, p. 1793–1801.

Shulman, G. I. (2000) Cellular mechanisms of insulin resistance. In : The Journal of Clinical Investigation, vol. 106, n° 2, p. 171–176.

Spencer, Michael; Yao-Borengasser, Aiwei; Unal, Resat; Rasouli, Neda; Gurley, Catherine M.; Zhu, Beibei et al. (2010) Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. In : American journal of physiology. Endocrinology and metabolism, vol. 299, n° 6, E1016-27.

Strissel, Katherine J.; DeFuria, Jason; Shaul, Merav E.; Bennett, Grace; Greenberg, Andrew S.; Obin, Martin S. (2010) T-cell recruitment and Th1 polarization in adipose tissue during diet-induced obesity in C57BL/6 mice. In : Obesity (Silver Spring, Md.), vol. 18, n° 10, p. 1918–1925.

Sun, Kai; Kusminski, Christine M.; Scherer, Philipp E. (2011) Adipose tissue remodeling and obesity. In : The Journal of Clinical Investigation, vol. 121, n° 6, p. 2094–2101.

Talukdar, Saswata; Oh, Da Young; Bandyopadhyay, Gautam; Li, Dongmei; Xu, Jianfeng; McNelis, Joanne et al. (2012) Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. In : Nature medicine, vol. 18, n° 9, p. 1407–1412.

Tardelli, Matteo; Zeyda, Karina; Moreno-Viedma, Veronica; Wanko, Bettina; Grün, Nicole G.; Staffler, Günther et al. (2016) Osteopontin is a key player for local adipose tissue macrophage proliferation in obesity. In : Molecular metabolism, vol. 5, n° 11, p. 1131–1137.

Tchkonia, Tamara; Thomou, Thomas; Zhu, Yi; Karagiannides, Iordanes; Pothoulakis, Charalabos; Jensen, Michael D.; Kirkland, James L. (2013) Mechanisms and metabolic implications of regional differences among fat depots. In : Cell metabolism, vol. 17, n° 5, p. 644–656.

Trayhurn, Paul (2013) Hypoxia and adipose tissue function and dysfunction in obesity. In : Physiological Reviews, vol. 93, n° 1, p. 1–21.

van Furth, R.; Cohn, Z. A. (1968) The origin and kinetics of mononuclear phagocytes. In : The Journal of experimental medicine, vol. 128, n° 3, p. 415–435.

van Furth, R.; Cohn, Z. A.; Hirsch, J. G.; Humphrey, J. H.; Spector, W. G.; Langevoort, H. L. (1972) The mononuclear phagocyte system. A new classification of macrophages, monocytes, and their precursor cells. In : Bulletin of the World Health Organization, vol. 46, n° 6, p. 845–852.

van Marken Lichtenbelt, Wouter D.; Vanhommerig, Joost W.; Smulders, Nanda M.; Drossaerts, Jamie M. A. F. L.; Kemerink, Gerrit J.; Bouvy, Nicole D. et al. (2009) Cold-activated brown adipose tissue in healthy men. In : The New England journal of medicine, vol. 360, n° 15, p. 1500–1508.

Vasanthakumar, Ajithkumar; Moro, Kazuyo; Xin, Annie; Liao, Yang; Gloury, Renee; Kawamoto, Shimpei et al. (2015) The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. In : Nature immunology, vol. 16, n° 3, p. 276–285.

Virtanen, Kirsi A.; Lidell, Martin E.; Orava, Janne; Heglind, Mikael; Westergren, Rickard; Niemi, Tarja et al. (2009) Functional brown adipose tissue in healthy adults. In : The New England journal of medicine, vol. 360, n° 15, p. 1518–1525.

Waki, Hironori; Tontonoz, Peter (2007) Endocrine functions of adipose tissue. In : Annual review of pathology, vol. 2, p. 31–56.

Wang, Youfa; Rimm, Eric B.; Stampfer, Meir J.; Willett, Walter C.; Hu, Frank B. (2005) Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. In : The American Journal of Clinical Nutrition, vol. 81, n° 3, p. 555–563.

Weisberg, Stuart P.; Hunter, Deborah; Huber, Reid; Lemieux, Jacob; Slaymaker, Sarah; Vaddi, Kris et al. (2006) CCR2 modulates inflammatory and metabolic effects of high-fat feeding. In : The Journal of Clinical Investigation, vol. 116, n° 1, p. 115–124.

Weisberg, Stuart P.; McCann, Daniel; Desai, Manisha; Rosenbaum, Michael; Leibel, Rudolph L.; Ferrante, Anthony W. (2003) Obesity is associated with macrophage accumulation in adipose tissue. In : The Journal of clinical investigation, vol. 112, n° 12, p. 1796–1808.

Winer, Daniel A.; Winer, Shawn; Shen, Lei; Wadia, Persis P.; Yantha, Jason; Paltser, Geoffrey et al. (2011) B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. In : Nature medicine, vol. 17, n° 5, p. 610–617.

Winer, Shawn; Chan, Yin; Paltser, Geoffrey; Truong, Dorothy; Tsui, Hubert; Bahrami, Jasmine et al. (2009) Normalization of obesity-associated insulin resistance through immunotherapy. In : Nature medicine, vol. 15, n° 8, p. 921–929.

Wu, Davina; Molofsky, Ari B.; Liang, Hong-Erh; Ricardo-Gonzalez, Roberto R.; Jouihan, Hani A.; Bando, Jennifer K. et al. (2011) Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. In : Science (New York, N.Y.), vol. 332, n° 6026, p. 243–247.

Wu, Jun; Boström, Pontus; Sparks, Lauren M.; Ye, Li; Choi, Jang Hyun; Giang, An-Hoa et al. (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. In : Cell, vol. 150, n° 2, p. 366–376.

Xu, Haiyan; Barnes, Glenn T.; Yang, Qing; Tan, Guo; Yang, Daseng; Chou, Chieh J. et al. (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. In : The Journal of Clinical Investigation, vol. 112, n° 12, p. 1821–1830.

Yin, Xiao; Lanza, Ian R.; Swain, James M.; Sarr, Michael G.; Nair, K. Sreekumaran; Jensen, Michael D. (2014) Adipocyte mitochondrial function is reduced in human obesity independent of fat cell size. In : The Journal of clinical endocrinology and metabolism, vol. 99, n° 2, E209-16.

Young, P.; Arch, J.R.S.; Ashwell, Margaret (1984) Brown adipose tissue in the parametrial fat pad of the mouse. In : FEBS letters, vol. 167, n° 1, p. 10–14.

Zamarron, Brian F.; Mergian, Taleen A.; Cho, Kae Won; Martinez-Santibanez, Gabriel; Luan, Danny; Singer, Kanakadurga et al. (2017) Macrophage Proliferation Sustains Adipose Tissue Inflammation in Formerly Obese Mice. In : Diabetes, vol. 66, n° 2, p. 392–406.

Zatterale, Federica; Longo, Michele; Naderi, Jamal; Raciti, Gregory Alexander; Desiderio, Antonella; Miele, Claudia; Beguinot, Francesco (2019) Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. In : Frontiers in Physiology, vol. 10, p. 1607.

Zhang, Y.; Proenca, R.; Maffei, M.; Barone, M.; Leopold, L.; Friedman, J. M. (1994) Positional cloning of the mouse obese gene and its human homologue. In : Nature, vol. 372, n° 6505, p. 425–432.

Zheng, C.; Yang, Q.; Cao, J.; Xie, N.; Liu, K.; Shou, P. et al. (2016) Local proliferation initiates macrophage accumulation in adipose tissue during obesity. In : Cell death & disease, vol. 7, e2167.

Zwick, Rachel K.; Guerrero-Juarez, Christian F.; Horsley, Valerie; Plikus, Maksim V. (2018) Anatomical, Physiological, and Functional Diversity of Adipose Tissue. In : Cell metabolism, vol. 27, n° 1, p. 68–83.

## 4.1 CD4+ T cells regulate glucose homeostasis independent of adipose tissue dysfunction in mice

Georg Brinker[1], Janine Froeba[1, 2], Lilli Arndt[2], Julia Braune[1, 2], Constance Hobusch[1], Andreas Lindhorst[1, 2], Ingo Bechmann[1], Martin Gericke[1, 2]

[1] Institute of Anatomy, Leipzig University, D-04103 Leipzig, Germany,

[2] Institute of Anatomy and Cell Biology, Martin-Luther-University Halle-Wittenberg, D-06108 Halle (Saale), Germany,

Key Words: Adipose tissue, inflammation, lymphocytes, macrophages, obesity

**No conflict of interest**

Correspondence to: Martin Gericke

Institute of Anatomy and Cell Biology, Martin-Luther-University Halle-Wittenberg,

Grosse Steinstrasse 52, D-06108 Halle (Saale), Germany

Phone: +49 345 557 1708, Fax: +49 345 557 1700

Email: martin.gericke@medizin.uni-halle.de

**Abbreviations:**

Adipose tissue – AT

Adipose tissue lymphocyte – ATL

Adipose tissue macrophage – ATM

Bromodeoxyuridine – BrdU

Crown-like structure – CLS

Forkhead box P3 – FOXP3

Glucose tolerance testing – GTT

High-fat diet – HFD

Insulin tolerance testing – ITT

Interferon-γ - IFNg

Normal chow diet – NCD

Osteopontin – OPN

Proliferating cell nuclear antigen – PCNA

Regulatory T cell – Treg

Suppression of tumorigenicity 2 – ST2

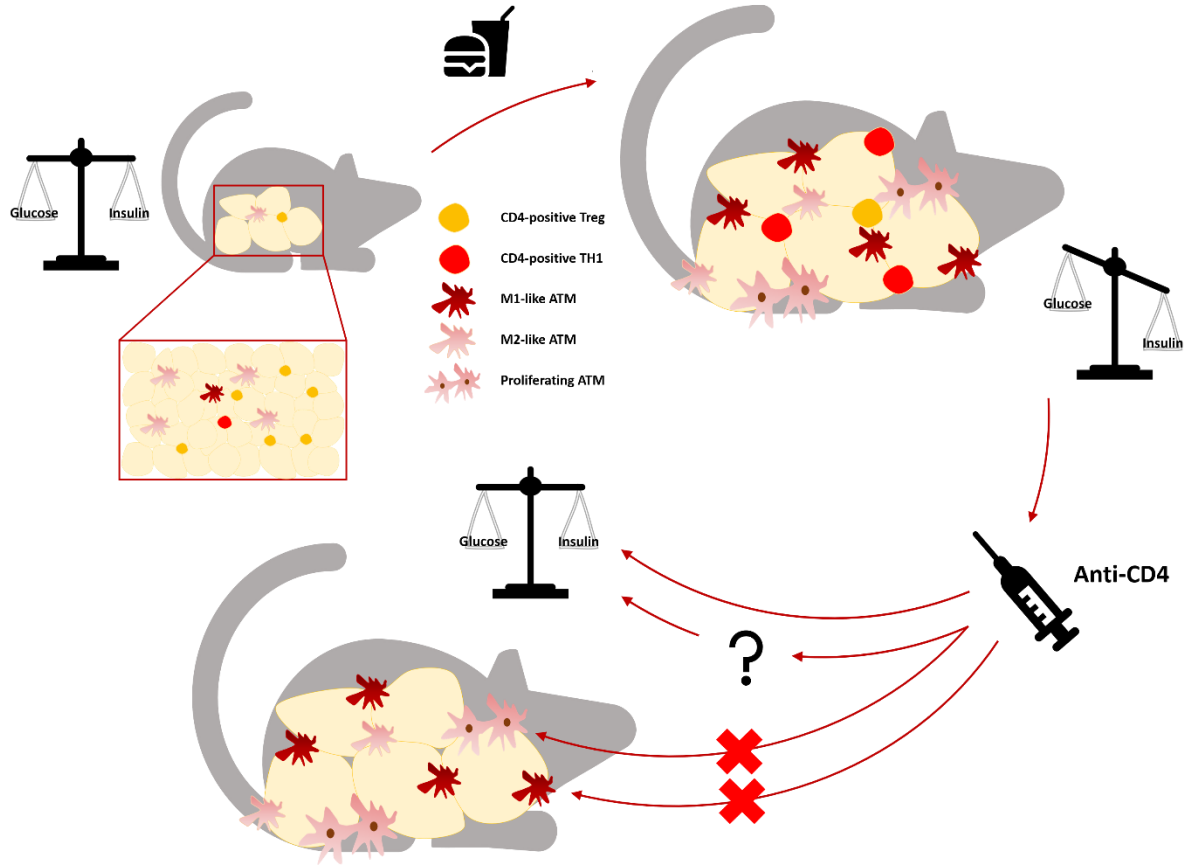
T helper type 2 cell – TH2 cell

Visceral adipose tissue – VAT

## Abstract

Obesity is frequently associated with a chronic low-grade inflammation in the adipose tissue (AT) and impaired glucose homeostasis. Adipose tissue macrophages (ATMs) have been shown to accumulate in the inflamed AT either by means of recruitment from the blood or local proliferation. ATM proliferation and activation can be stimulated by TH2 cytokines, such as IL-4 and IL-13, suggesting involvement of CD4-positive T cells in ATM proliferation and activation. Furthermore, several studies have associated T cells to alterations in glucose metabolism. Therefore, we sought to examine a direct impact of CD4-positive T cells on ATM activation, ATM proliferation and glucose homeostasis using an *in vivo* depletion model. Surprisingly, CD4 depletion did not affect ATM activation, ATM proliferation or insulin sensitivity. However, CD4 depletion led to a significant improvement of glucose tolerance. In line with this, we found moderate disturbances in pancreatic endocrine function following CD4 depletion. Hence, our data suggest that the effect on glucose metabolism observed after CD4 depletion might be mediated by organs other than AT and independent of AT inflammation.


## Graphical Abstract



## Introduction

Obesity has become an increasing burden for health systems around the world and has led amongst others to an unrivalled upsurge of type 2 diabetes (James 2008). The socioeconomic and personnel effort to tackle the consequences of the obesity epidemic have become enormous (Economic Costs of Diabetes in the U.S. in 2017 2018). It is therefore pivotal to elucidate the molecular foundation of obesity in order to develop targeted therapies.

On a cellular level, obesity is associated with a chronic low-grade inflammation in the adipose tissue (AT) that is characterized by an accumulation of various immune cells, namely T cells, B cells, neutrophils, mast cells and macrophages (Weisberg et al. 2003; Winer et al. 2011; Winer et al. 2009; Liu et al. 2009; Talukdar et al. 2012). This inflammation is most pronounced in the visceral adipose tissue (VAT), which is believed to have a more detrimental effect on metabolism than subcutaneous adipose tissue (SAT) (O'Rourke et al. 2009; Gastaldelli et al. 2002). Macrophages constitute the largest proportion of immune cells in the AT of obese rodents and humans respectively (Weisberg et al. 2003), and the extent of the accumulation of macrophages in the AT seems to be closely correlated to the degree of insulin resistance (Klöting et al. 2010). Macrophages occur in a continuum of different phenotypes from which two types are generally discerned: M1 macrophages are induced by pro-inflammatory cytokines such as interferon-γ (IFNg) and express CD11c as a surface marker (Cho, Morris et Lumeng 2014; Martinez et Gordon 2014). In contrast, activation of M2 macrophages occurs in a TH2-related microenvironment, particularly due to interleukin 4 (IL-4) and IL-13, and leads to expression of CD206 and CD301 (Martinez et Gordon 2014; Cho, Morris et Lumeng 2014). Both populations seem to increase in number in the AT during obesity, but there is a marked increase in the proportion of M1 macrophages (Lumeng, Bodzin et Saltiel 2007), which are located preferentially around dead adipocytes, in so-called crown-like structures (CLS). Further, it has been shown that the accumulation of adipose tissue macrophages (ATMs) during obesity occurs either by means of monocyte recruitment from the blood or proliferation of resident ATMs (Zheng et al. 2016). We and others have recently shown that the number of proliferating ATMs increases during obesity (Bourlier et al. 2008; Amano et al. 2014; Haase et al. 2014) and that this proliferation is at least partially mediated by cytokines such as IL4, IL13 and osteopontin (OPN), which are related to CD4-positive T helper cells (TH2 cells) (Braune et al. 2017; Tardelli et al. 2016; Shirakawa et al. 2016). This observation might suggest a link between macrophage activation and proliferation and CD4-positive TH2 cells.

CD4-positive T cells belong to the network of adaptive immune cells, which seem to play an important role in obesity-induced inflammation and insulin resistance (Osborn et Olefsky 2012). To what extent the effect of lymphocytes on metabolism is immediate or mediated by

macrophages and other immune cells is not known. Studies on mice with a RAG1 knockout, which abrogates the rodents capacity to produce any type of lymphocyte, have shown worsening of metabolic parameters, suggesting a protective role of lymphocytes (Winer et al. 2009). It is assumed that the phenotype and function of lymphocytes alters during the course of obesity, leading to a loss of their protective properties. In line with this, depletion of CD3-positive T cells, CD20-positive B cells and CD8-positive T cells in HFD-fed mice have shown attenuation of AT inflammation and improvement of metabolic parameters (Winer et al. 2009; Winer et al. 2011; Nishimura et al. 2009). More accurately, depletion of CD3-positive T cells led to a marked upregulation of CD206-expressing ATMs while leaving adipocyte diameter unchanged. Likewise, depletion of CD8-positive T cells resulted in a decrease of CD11c-positive ATMs and a significant decrease of CLS formation. Both studies hence bring to attention the association between T-cell-mediated changes in glucose metabolism on one side and T-cell-mediated changes in VAT microenvironment on the other (Winer et al. 2009; Nishimura et al. 2009). The effect of an *in vivo* CD4 cell depletion on AT inflammation and glucose homeostasis during obesity is as yet undescribed, although it has been suggested that certain distinct CD4-positive cell populations might contribute to immune-mediated alterations in AT inflammation and glucose metabolism (Shirakawa et al. 2016; Feuerer et al. 2009). Among these, VAT regulatory T cells (Tregs) have recently gained considerable attention for their potential to regulate AT and metabolic homeostasis (Zeng et al. 2018). In line with this, numerous studies have demonstrated that *in situ* expansion of Treg population in VAT led to attenuation of AT inflammation and improvement of glucose metabolism (Vasanthakumar et al. 2015; Han et al. 2015b; Li et al. 2018; Feuerer et al. 2009; Zeng et al. 2018). Importantly, *in vivo* loss-of-function models of VAT Tregs failed to show an effect on glucose metabolism in HFD-fed mice (Deng et al. 2017; Cipolletta et al. 2012; Bapat et al. 2015; Zeng et al. 2018).

CD4 cells, analogous to other immune cells in the AT, seem to increase in absolute numbers during obesity, yet inconsistencies exist as for the proportional changes: Some authors argue for a relatively stable CD4 population (Strissel et al. 2010), whereas others suggest a proportional diet-dependent decrease of CD4 cells in the course of obesity (Nishimura et al. 2009; Winer et al. 2009). Evidence further points towards a phenotypic switch among CD4 cells in the inflamed AT with a growing IFNg-positive population (Strissel et al. 2010; Winer et al. 2009) and a loss of forkhead box P3- (FOXP3) and suppression of tumorigenicity 2- (ST2) positive cells during obesity (Cipolletta et al. 2015; Bapat et al. 2015; Han et al. 2015a). Both FOXP3 and ST2 are generally believed to have anti-inflammatory properties in the context of obesity induced inflammation and various experiments suggest a beneficial effect on glucose metabolism (Feuerer et al. 2009; Miller et al. 2010; Eller et al. 2011; Han et al. 2015a;

Vasanthakumar et al. 2015). The loss of these cells hence suggests the notion of a CD4 population that acquires a detrimental effect on glucose metabolism during the development of obesity.

The present work sought to elaborate further on the *modus operandi* of CD4-positive cells in the AT and aimed to elicit a possible impact on ATM activation and proliferation. We therefore established 3-day and 2-week CD4 depletion protocols to evaluate short-term and potentially more direct changes as well as long-term effects on changes to the microenvironment. We show that short-term and long-term CD4 depletion have no apparent influence on either macrophage activation or macrophage proliferation in the AT, yet seem to have an impact on glucose homeostasis as measured by glucose tolerance testing (GTT). Furthermore, we show that long-term depletion of CD4-positive T cells causes dysregulation of the pancreatic endocrine axes, possibly contributing to the altered glucose metabolism following CD4 depletion. We conclude that in our study systemic CD4 depletion was shown to have no apparent effect on either CLS formation, ATM proliferation or activation, yet entailed beneficial glucose tolerance potentially due to reasons beyond effects on VAT inflammation.

## Results

*CD4-positive T cells show time- and diet-dependent changes in the inflamed AT*

First, we performed immunofluorescence staining of CD3- (Fig. 1B) and CD4- (Fig. 1C) positive T cells to establish their presence and evaluate their distribution in the murine AT. We observed that CD3- and CD4-positive cells were mainly present in the CLS. Using flow cytometry, we then aimed to phenotype the CD3- / CD45-positive T cell population into their CD4- and CD8-expressing subsets. Therefore, we analysed VAT of mice fed either a HFD or a NCD for 4, 12 or 24 weeks. We detected a significant proportional increase of CD4-positive cells over time and independent of diet (Fig. 1A). At 24 weeks, the proportion of CD8-positive cells was significantly larger in HFD-fed compared to control animals. This diet-dependent difference was absent in the overall proportion of CD4 cells (Fig. 1A). However, further phenotyping of the CD4-positive subset revealed significant diet-dependent changes of CD4 subsets. IFNg-positive CD4 cells significantly dropped at 12 weeks and then seemed to increase after 24 weeks in the HFD-fed animals (Fig. 1D). In contrast, expression of both Treg-associated FOXP3 and TH2-associated ST2 among CD4-expressing lymphocytes showed a marked increase until 12 weeks in both groups followed by a further increase in NCD-fed mice and a decrease in HFD-fed mice (Fig. 1E and F). At 24 weeks, expression of both ST2 and FOXP3 were significantly lower in the HFD-fed group compared to NCD-fed controls (Fig. 1E and F). This reduced ST2 expression in obese mice at 24 weeks was observed in both FOXP3-positive and FOXP3-negative cells, but was more pronounced in the FOXP3-negative population (Fig. 1G and H).

*3-day depletion of CD4-positive cells does not alter ATM activation or proliferation*

To study the direct impact of CD4 cells on AT inflammation, we established a 3-day depletion protocol. CD4 specific antibodies were shown to efficiently deplete CD4-positive T cells by ~99%. We further showed that depletion of CD4-positive T cells was consistent in both adipose and splenic tissue (Fig 2C and D). Further, depletion did not result in significant weight loss averaging ~1g in both groups after 3 days of depletion (data not shown).

After HFD-feeding for 24 weeks and antibody treatment according to protocol, we analysed the AT phenotype. Fat cell expansion and formation of CLS are signs of tissue remodeling under high-caloric diet and could be observed in all animals (Fig. 3A and B). However, AT morphology as measured by adipocyte diameter did not alter between T-cell-depleted mice and mice treated with an appropriate isotype control (Fig. 3E). In order to evaluate AT inflammation, we counted the number of CLS in relation to adipocytes, which also revealed no significant difference (Fig. 3F). Another marker for the progression of AT inflammation is the

proportion of ATMs among all stromal vascular cells. Again, no difference was observed (Fig. 3H). ATM activation as measured by the ratio of CD11c-positive ATMs (classically activated or M1 macrophages) in relation to CD206-expressing ATMs (alternatively activated or M2 macrophages) was quantified using flow cytometry and showed no effect under CD4 cell depletion (Fig. 3I). We then went on to assess a possible impact of CD4 depletion on ATM proliferation. Firstly, we stained VAT for the proliferation marker proliferating cell nuclear antigen (PCNA; Fig. 3C and D). Since CLS have been shown to be the primary site of ATM proliferation, we focused our analysis on cells residing within these structures. Quantification of immunohistochemistry showed no significant changes under CD4 cell depletion (Fig 3G). Secondly, we performed flow cytometric analysis of Bromodeoxyuridine (BrdU) staining (Fig. 3J) which in accordance with the immunohistochemistry showed no significant difference between the groups. Likewise, mRNA levels of genes associated with ATM proliferation or activation showed no significant changes under CD4 cell depletion (Fig. 3K).

In the next step, we intended to compare the impact of CD4 cell depletion, as described above, with the effect of total T cell depletion. For that reason, we designed a similar 3-day depletion protocol for CD3 cells. However, we became aware that some mice in the CD3-depleted cohort showed signs of diarrhea and peritoneal inflammation in conjunction with a significant weight loss when compared with their IgG-injected control littermates (Fig. S2N). The depletion itself showed a moderate depletion efficiency of ~66% in both spleen and VAT (Fig. S1). Importantly, ATM proliferation and activation remained unaffected by CD3 depletion (Fig. S2). Due to the observed weight loss and inflammatory changes however, we concluded that the interpretation of our data was severely biased and decided to show these results in the supplementary section only.

*2-week depletion of CD4-positive cells does not alter ATM activation or proliferation*

In order to rule out any indirect long-term effect of CD4-positive lymphocytes on ATM proliferation and activation, we established a 14-day depletion protocol. We then assessed AT integrity by means of immunohistochemistry as described in the previous section. Similar to the 3-day depletion experiment, AT morphology showed no marked difference after 14 days of CD4 depletion compared to the isotype control (Fig. 4A and B). Likewise, adipocyte diameter and CLS density did not differ significantly between CD4-depleted and control mice (Fig. 4E and F). Quantification of proliferation by means of PCNA staining (Fig. 4C, D and G) and by means of flow cytometry (Fig. 4J) also showed no alteration between the two groups, as did the proportion of ATMs and M1 to M2 ratio (Fig. 4H and I). Finally, the study of mRNA associated

with ATM proliferation and activation revealed no significant changes in T-cell-depleted murine AT (Fig. 4K-N).

*Glucose tolerance is improved under CD4-specific antibody treatment*

In the next step, we aimed to determine any effect of CD4-specific antibody treatment on glucose metabolism. We therefore performed GTT and ITT as described above. Interestingly, during GTT we observed significantly lower glucose levels at 30 minutes and 120 minutes after glucose injection among the T-cell-depleted mice compared to mice treated with an appropriate isotype control (Fig. 5A). To evaluate the metabolic relevance, we performed area-under-the-curve analysis which also showed a significant difference between the two groups (Fig. 5B), suggesting a beneficial effect of CD4 cell depletion on glucose tolerance. During insulin tolerance testing we observed a mild, yet not significant, trend towards improvement of insulin tolerance under CD4 depletion (Fig. 5C and D).

*Pancreatic endocrine function is altered following CD4 depletion*

Finally, we sought to elaborate on a possible contribution of the pancreas to the observed difference in glucose metabolism. Morphometric analysis of pancreatic tissue as measured by islet quantity and size (Fig. 6B and C, representative images in Fig. 6A) revealed no significant alterations in mice injected with CD4-depleting antibody. However, frequency distribution analysis revealed a significantly higher proportion of very small islets (40 – 60 µm) in pancreata of CD4-depleted mice (Fig. 6D). We then aimed at assessing the pancreatic endocrine function under CD4 depletion. Interestingly, fasting plasma insulin appeared to be similar between the two groups (Fig. 6E). Likewise, pancreatic insulin content was unchanged following CD4 depletion (Fig. 6G). In contrast, we observed a significant decrease in whole pancreatic content of glucagon (Fig. 6H). Further, protein analysis normalised to the islet protein content, as measured by GLUT2, also revealed a relative increase of somatostatin (Fig. 6I).

## Discussion

The role of T cells in AT inflammation has been extensively studied over the past two decades. Two major *in vivo* depletion studies have suggested a substantial effect of adipose tissue lymphocytes (ATLs) on AT integrity and glucose homeostasis: CD3 depletion was shown to reduce M1 and increase M2 ATMs in VAT and positively influence glucose homeostasis (Winer et al. 2009). Likewise, depletion of CD8-positive T cells resulted in an increased M2/M1 ratio in VAT, a reduction in CLS density and beneficial effects on insulin sensitivity and glucose tolerance (Nishimura et al. 2009). These two studies both suggest that ATLs acquire a pro-inflammatory phenotype during high-fat feeding, depletion of which partially restores the disrupted AT integrity and attenuates the impaired glucose metabolism in HFD-fed mice. In this study we aimed to substantiate this claim by elucidating the effects of CD4-positive T cells on AT inflammation and glucose homeostasis.

There are conflicting propositions in the literature, firstly, as to the sequence of events regarding T cell involvement and, secondly, as to the extent of quantitative and qualitative changes in the VAT T cell population. Studies using mRNA as a target have suggested a relatively early expansion of CD3-positive cells in HFD-induced inflammation that coincides with changes in glucose homeostasis and precedes macrophage expansion (Deng et al. 2013; Kintscher et al. 2008). It has been argued that this early expansion of CD3-positive cells in VAT is limited to the CD8-positive subset whereas CD4-positive T cells decrease (Nishimura et al. 2009; Winer et al. 2009). However, other studies make the case for an early CD4 cell expansion (Shirakawa et al. 2016) or a relatively stable CD4 population (Strissel et al. 2010). In our study, we demonstrate an age- but not diet-dependent proportional increase of CD4 cells in VAT and a numerically but not phenotypically stable CD4 population with regard to the impact of a HFD. Equally unclear is the role of IFNg-releasing CD4 cells with studies arguing for either an increase (Winer et al. 2009) or a reduction (Zamarron et al. 2017) of this population under the influence of HFD-feeding. Our results seem to concur with the latter, although we also detected an increase to lean levels at a later time point. Our data thus argue against a major impact of IFNg released by CD4 cells in the context of late AT inflammation. However, the highly significant drop of IFNg-releasing CD4 cells at 12 weeks of HFD also described by Zamarron et al. (Zamarron et al. 2017) poses the question of different AT responses to high-caloric stimuli during different time points and could instigate further research. Relative consensus exists as to the FOXP3-positive Treg population that seems to increase with age and decrease under high-caloric feeding (Feuerer et al. 2009; Nishimura et al. 2009; Bapat et al. 2015; Cipolletta et al. 2015; Winer et al. 2009) selectively in VAT but not splenic tissue or SAT (Feuerer et al. 2009). A similar dynamic has been reported for ST2 expression on FOXP3-positive and FOXP3-negative T cells respectively (Han et al. 2015a). Our data agree with this observation,

leading us to argue for an obesity-induced decrease of FOXP3 and ST2 expression that is inverse to the age-related increase in chow-fed animals. ST2 and FOXP3 are generally considered to have anti-inflammatory properties in the context of obesity-induced inflammation (Eller et al. 2011; Han et al. 2015a; Feuerer et al. 2009; Miller et al. 2010; Vasanthakumar et al. 2015).

Our results hence led us to believe that depletion of the CD4 population that has tilted towards a more pro-inflammatory phenotype would propel similar changes as seen with CD8 or CD3 depletion respectively. We therefore established a highly efficient 3-day and 14-day depletion protocol to account for short- and long-term effects on AT integrity. It should be noted that the mice cohorts used in both experiments differed quite significantly regarding their total body weight. However, the two experiments were not designed to be compared directly and both groups express a degree of AT inflammation that is distinct from AT morphology in lean animals. Interestingly, both depletion experiments showed no effect of depletion on adipocyte size, number of CLS, macrophage activation or proliferation. We were able to reproduce these results using a short-term CD3 depletion protocol, however interpretation of the data was confounded by weight loss in depleted mice.

It should be acknowledged that weight loss has been reported in numerous studies using the CD3-depleting hamster IgG 145 2C11 (Ferran et al. 1990; Ferran et al. 1991; Alegre et al. 1990; Winer et al. 2009; Vossen et al. 1995; Vossen et al. 1994). This has been attributed to a cytokine release syndrome triggered by activation of Fc receptors. However, in this study we used the rat IgG 17A2 which has been explicitly demonstrated not to induce the same significant morbidity associated with the 145 2C11 antibody (Vossen et al. 1995; Vossen et al. 1994). The severe reaction of some of the mice in our anti-CD3 cohort was therefore unexpected. Caution in using and interpreting data obtained from CD3 depletion with both, 17A2 and 145 2C11, should therefore be advised. However, for appropriate comparison between data after CD3 and CD4 depletion, a similar IgG based approach has been used, which did not show any adverse effects in CD4 depleted mice.

We and others have recently described ATM proliferation as a characteristic of AT inflammation (Amano et al. 2014; Haase et al. 2014), which seems to be dependent on cytokine stimulation namely by TH2-related cytokines IL-4, IL-13 and OPN (Braune et al. 2017; Tardelli et al. 2016). Furthermore, CD4 cells have been shown to be the main source of OPN in VAT (Shirakawa et al. 2016). However, various techniques used in this study to evaluate ATM proliferation have been consistent in showing no effect of CD4 depletion, leading us to believe that CD4 cells play a minor role in prompting ATM proliferation under high-caloric feeding.

CD4 depletion however seems to have some effect on glucose metabolism, since we showed significant improvement of GTT, but not ITT following CD4 depletion. This suggests some degree of impaired insulin production in reaction to glucose challenge but not insulin resistance *per se*. This discrepancy led us to hypothesize a potential pancreatic involvement. Both groups showed signs of islet hypertrophy, which have been described as characteristic for diet-induced obesity (Roat et al. 2014). Yet, we failed to observe a significant difference in trophic conditions under CD4 depletion. Furthermore, we did neither detect an increase of insulin expression nor enhanced fasting insulin levels under CD4 depletion. Interestingly however, we observed a significant reduction of glucagon in the pancreatic tissue and a significant increase of somatostatin in proportion to islet cell mass in CD4-depleted mice, suggesting an impact of CD4 cells on alpha and delta cell homeostasis. Alpha-cell dysfunction has been described as a hallmark of type 2 diabetes (Müller et al. 1970; Gromada, Chabosseau et Rutter 2018). Indeed, hyperglucagonism was shown to be present in obese subjects in the fed and fasted state in numerous studies (Newgard et al. 2009; Stern et al. 2019; Knop et al. 2012; Kellard et al. 2020). Furthermore, alpha cell dysfunction seems to result in an inability to suppress glucagon in reaction to glucose challenge (Bagger et al. 2014; Wagner et al. 2017; Merino et al. 2015). Novel therapeutics rely partly on the antagonism of glucagon action (Hædersdal et al. 2018). The inadequate alpha cell output, in turn, appears to be counter-regulated by somatostatin (Kellard et al. 2020; Strowski et al. 2000). It has been suggested that this effect is mediated by an absolute decrease in delta cell output and a loss of somatostatin sensitivity of alpha cells in the state of impaired glucose tolerance. (Omar-Hmeadi et al. 2020; Kellard et al. 2020) Therefore, it might be reasonable to speculate that glucagon and somatostatin disturbance, as observed in our study, partly contribute to the beneficial metabolic phenotype exhibited by CD4-depleted mice. However, studying the pancreatic involvement was not the primary focus of this study and should therefore warrant further research.

Likewise, the beneficial effect of CD4 depletion on whole body glucose tolerance is in agreement with the notion that CD4 cells acquire a phenotype which is detrimental to insulin sensitivity during the course of obesity. However, whereas CD3 and CD8 depletion seem to influence both glucose homeostasis and ATM activation, effects of CD4 depletion seem to be independent of such changes. Potentially these effects are mediated by cytokines that do not have a major impact on either ATM phenotype or proliferation.

That said, in the absence of an established method to selectively deplete organ-specific cells *in vivo* without prior genetic modifications, we currently rely on systemic depletion protocols, making it difficult to attribute an observed systemic effect to site-specific changes in the microenvironment. Such inherent limitations apply to all depletion studies and therefore it has to be highlighted that this study was not designed to provide a mechanistic insight into the origins

of CD4-mediated effects on glucose metabolism and that the above mentioned models postulating a pancreatic or VAT involvement in CD4-mediated changes in glucose tolerance are speculative in nature and do not rule out the potential involvement of other organs.

In conclusion, our results show that the CD4 cell population in VAT shifts towards a more pro-inflammatory phenotype and that systemic depletion of CD4 cells improves glucose tolerance while leaving insulin sensitivity, AT morphology, ATM activation and ATM proliferation as signs of AT dysfunction unaffected.

## Material and Methods

### Experimental Animals

Mice strains were kept in our local animal facility in a temperature controlled, 12h-light/dark cycle environment with *ad libitum* access to food and water. In order to induce obesity C57BL/6 mice were fed a high-fat diet (60%kcal fat; Ssniff Spezialdiäten, Soest, Germany) or a normal chow diet (9% kcal fat; Ssniff Spezialdiäten) up to 24 weeks, starting at 6 weeks of age. For some experiments, CSF1R-eGFP$^{+/-}$ (MacGreen; (Sasmono et al. 2003)) or LysMCre x TDTO$^{flx/flx}$ (Orthgiess et al. 2016) on a C57BL/6 background were used, depending on the spectral requirements. Of note, reporter mice develop an AT inflammation similar to wild-type C57BL/6 mice (Gericke et al. 2015). All studies used males and were approved by the local ethics committee (accreditation number 42502-2-1554 MLU).

### Depletion

For depletion of CD4-positive cells, mice were injected with 120 µl of anti-CD4 (GK 1.5; Bio-Legend, San Diego, CA) or an isotype control (RTK4530; BioLegend) using a 3-day protocol and a 2-week protocol. With the 3-day depletion protocol, mice received either injection for 3 consecutive days. With the 2-weeks protocol, mice received 3 injections each week for two weeks as described by Nishimura et al. (Nishimura et al. 2009). ITT and GTT were performed at the end of the 2-week depletion protocol only. Experiments were performed under specific pathogen free (SPF) conditions, following FELASA criteria. Importantly, mice after CD4 depletion were indistinguishable from control mice, without any signs of diarrhea, conjunctivitis or a significant drop in body weight.

For depletion of CD3-positive cells, mice were injected with 120 µl of anti-CD3 (17A2; Bio-Legend, San Diego, CA) or an isotype control (RTK4530; BioLegend) using a 3-day protocol as described above. Strikingly however, some mice showed signs of diarrhea and peritoneal inflammation in association with a considerable weight loss (Fig. S2N) following CD3 cell depletion, for which reason, we have decided to show the acquired data in the supplementary section only (Fig. S1 and 2).

### ITT/GTT

For ITT, baseline glucose level was measured before mice were injected with insulin (Insuman Rapid, 100 IU/ml). Blood glucose was measured again at 15, 30 and 60 min after injection. Mice received 1.5 U/kg insulin solution intraperitoneally. ITT was performed 3 days prior to GTT. For GTT, mice were fasted for 12 h prior to testing. Baseline sugar was measured prior

to injection of glucose (20%; B. Braun, Melsungen, Germany). Glucose level was then measured at 15, 30, 60 and 120 min after injection. Mice received 1g/kg glucose solution intraperitoneally.

**Immunofluorescence**

Mice were sacrificed and VAT was dissected. VAT was then fixed in zinc formalin overnight and embedded in paraffin. Paraffin sections were deparaffinized, microwaved and washed in PBS with 0.3% Triton (PBST). Subsequently, unspecific binding sites were blocked using PBST and 1% BSA for 1 h at room temperature. AT was stained with primary antibodies at 4°C overnight. For AT analysis, AT was stained using the macrophage marker Mac-2 (CL8942AP; 1:1000, Cedarlane; Burlington, Canada) and the fat cell marker Perilipin A (ab3526; 1:200; Abcam, Cambridge, U.K.). For proliferation studies, macrophages were stained against Mac-2 and the proliferation marker PCNA (ab15497; 1:200; Abcam). DAPI (1:10,000; Thermo Fisher Scientific, Schwerte, Germany) was used for nuclear staining. Appropriate secondary antibodies were selected and incubated for 1 h at room temperature. For AT analysis, images were taken using the Olympus BX40 epifluorescence microscope. For proliferation studies, AT was studied using an inverted confocal microscope (FV1000 Olympus, Hamburg, Germany).

**Analysis of AT Sections**

For AT analysis, the tissue was stained for Mac-2 and Perilipin A as described above. AT integrity was evaluated by measuring fat cell diameter and formation of CLS. CLS were defined as adipocytes entirely surrounded by leucocytes as described by others (Cinti et al. 2005; Strissel et al. 2007). CLS per field were counted in 10 randomly chosen fields on one section per animal. For the study of proliferating ATMs, staining for PCNA was performed as described above. ATMs were visualised by staining for Mac-2. Since it has been shown that up to 90% of ATMs in the inflamed AT reside in CLS (Cinti et al. 2005) and proliferation was shown to occur predominantly in CLS (Haase et al. 2014), 20-30 photographs of CLS were taken per section and analysed for content of proliferating cells.

**Analysis of pancreatic tissue sections**

For analysis of islet morphology, pancreatic tissue was stained with hematoxylin and eosin. Subsequently, the area of pancreatic tissue was determined in three HE stained sections per animal (minimal distance 300 µm). Islets were counted in these sections (mean 76.8$\pm$7.8 islets

per mouse) and displayed as number per $mm^2$ of whole pancreatic tissue. Similarly, the diameter of islets was measured and averaged over all islets present in all three sections. Images were taken using a Keyence microscope (Neu-Isenburg; Germany).

**Whole mount staining**

Whole mounts of AT from tdTomato (TDTO) reporter mice were used to visualise presence of CD3- and CD4-positive cells in AT. For that purpose, epididymal AT was immediately fixed after sacrifice for 20 min in zinc formalin (Polysciences, Hirschberg, Germany), washed in PBS and cut into small pieces (<1 $mm^3$). These AT pieces were then washed in PBS, blocked with staining buffer (3% bovine serum albumin (BSA) in PBS) for 1 hour at room temperature and stained with pre-labeled antibodies in staining buffer (anti-CD3-PE-Cy7; 1:100 145-2C11; BioLegend or anti-CD4-AF647; 1:100; RM4-5; BioLegend) overnight. We used Hoechst (1:10,000 in PBS; Life Technologies) to stain the nuclei. AT pieces were then washed three times in PBS and subsequently transferred into cavities of microscope slides and mounted using Fluorescence Mounting Medium (Dako; Hamburg; Germany). AT was studied using the Olympus FV1000 confocal microscope.

**Flow cytometry**

For flow cytometry analyses, VAT and spleen were dissected. VAT was digested with collagenase type II (Worthington Biochemical, Lakewood, NJ) centrifuged and the lipid phase discarded in order to gain a cell suspension of stromal vascular cells (SVCs). This suspension was then filtered through a 75 µm mesh. Splenic tissue was minced using a razor blade and pressed through a 70 µm mesh using a syringe plunger. Fc receptors of splenic cells and SVCs were blocked using anti-CD16/32 (1:100; eBioscience, Frankfurt, Germany) for 10 min on ice. Surface staining was performed using anti–CD45-FITC (30-F11; 1:100) or anti-CD45-APC-eFluor780 (30-F11, 1:200), anti–F4/80-PE-Cy7 (BM8, 1:100), anti–CD11c-PE (N418; 1:100; all eBioscience), anti–CD206-AF647 (MR5D3; 1:50; AbD Serotec, Kidlington, U.K.), anti-CD3-PE-Cy7 (145-2C11) anti-CD4-PE (RM4-5) or anti-CD4-AF647, (RM4-5), anti-CD8-AF647 (YTS156.7.7; all 1:100; all BioLegend) and anti-ST2-PE (RMST2-2; 1:100; eBioscience) for 20 min on ice. After fixation and permeabilization with the allophycocyanin (APC) BrdU flow kit (BD Pharmingen, Heidelberg, Germany) cells were again stained using anti-FOXP3-eF450 (FJK-16; 1:100; ThermoFisher), anti-IFN-gamma-PE (XMG1.2; 1:100; eBioscience) or anti–BrdU-Alexa Fluor 647 (PRB-1; 1:50; Abcam). For detection of BrdU, mice were injected with 200 µl BrdU 3 h prior to the experiment. After Fc-blocking, staining of surface markers and fixation as described above, cells were further treated with DNase IV (Sigma-

Aldrich, Deisenhofen, Germany). For IFNg detection, SVCs were cultured in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal calf serum and 0.6% PSA (penicillin, streptomycin und amphotericin B) at 5% $CO_2$/21% $O_2$ and 37˚C. Later, leucocyte activation cocktail with BD GolgiPlug™ (BD Pharmingen) was applied according to the manufacturer's protocol and the cells were incubated overnight for 12 h. The following day, Fc-blocking, staining and fixation were performed as described above. 7-aminoactinomycin D (7-AAD; BD Pharmingen) was used for DNA staining. Appropriate isotype controls were duly performed for all experiments. Analysis was performed using an LSR II (BD Pharmingen) equipped with FACSDiva software 8.0. Quantification of flow cytometry data was implemented using FlowJo software 10.0.5 (Tree Star, Ashland, OR). Gating strategies are described in detail in the supporting information (Fig. S3). Guideline for the use of flow cytometry studies were duly followed (Cossarizza et al. 2017).

**Gene expression analysis**

Analysis of relative gene expression was performed using quantitative real-time PCR (Maxima SYBR Green quantitative PCR master mix; Thermo Fisher Scientific) on a Bio-Rad CFX96 Manager system (Bio-Rad, Munich, Germany). RNA was extracted (TRI Reagent solution; Thermo Fisher Scientific), followed by synthesis of cDNA using oligo (dT) primers and a ProtoScript first-strand cDNA synthesis kit (New England Biolabs, Frankfurt am Main, Germany). Gene-specific primers (presented in Supplemental Table I) were designed with the Primer 3 software. Gene of interest mRNA levels were measured in duplicate and normalised to TATA-binding protein (TBP). The acquired data were analysed with the ΔΔCt method by Pfaffl (Bustin 2004).

**Enzyme-linked immunosorbent assay (ELISA)**

Plasma concentrations of insulin were measured using immunosorbent enzyme-linked assay according to the manufacturer's guideline (Mouse Insulin ELISA, ALPCO, Salem, USA).

**Western blot analysis**

Western blot was performed as described by others (Zibolka et al. 2020). Total protein from pancreas samples were extracted using RIPA lysis and extraction buffer (Thermo Fisher Scientific) supplemented with protease and phosphatase inhibitor cocktail (Roche, Basel, Switzerland). Equal amounts of protein (30 µg) were loaded to SDS-PAGE and transferred to nitrocellulose membrane. After blocking with 1% ROTI®Block (Carl Roth, Karlsruhe, Germany)

or 5% dry milk in TBS/T, blots were incubated with primary antibodies against insulin (1:1000, STJ24210; St John‘s Laboratory, London, United Kingdom), glucagon (1:500, PA5-13442; Thermo Fisher Scientific), somatostatin (1:2000, STJ95730; St John’s Laboratory), GLUT2 (1:4000, 07-1402, Merck Millipore, Burlington, USA) and GAPDH (1:1000, 3686, Cell Signaling Technology, Boston, USA). Followed by an incubation with horseradish peroxidase-conjugated secondary antibody, immunoreactions were detected by visualizing the peroxidase activity with an ECL Kit (Pierce™ ECL Western Blotting Substrate, Thermo Fisher Scientific). For reloading the membrane with primary antibody, blots were stripped with western blot stripping buffer (Thermo Fisher Scientific) according to the manufacturer’s instruction.

**Statistical analysis**

Data are presented as means ± SEM and as column bar graphs or as pie charts of at least three animals evaluated by the Student’s t-test, or the Mann-Whitney U test as calculated by GraphPad Prism (GraphPad Software, La Jolla, CA). A p-value $<0.05$ was considered statistically significant.

**Acknowledgements**

This work is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 209933838 – SFB 1052 (project B09). Martin Gericke is the recipient of this funding. We thank Kathrin Jäger and Andreas Lösche from the FACS core unit and Michaela Kirstein (MLU Halle) for technical assistance. Further, we thank Juliane Zibolka and Ivonne Bazwinsky-Wutschke (MLU Halle) for help with establishing Western Blot analyses.

**Author Contributions**

M.G. and I.B. designed the study. G.B. carried out the experiments with help from J.F. (ITT/GTT) and L.A. (ELISA, Western Blot), J.B. (qPCR; FACS), C.H. (genotyping) and A.L. (immunofluorescence analyses). G.B. analysed the data with help from J.F. and M.G. G.B. drafted and M.G. and I.B. revised the paper. All authors approved the final version of the manuscript.

**Conflict of interest**

The authors declare no financial or commercial conflict of interest.

**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Figure legends:

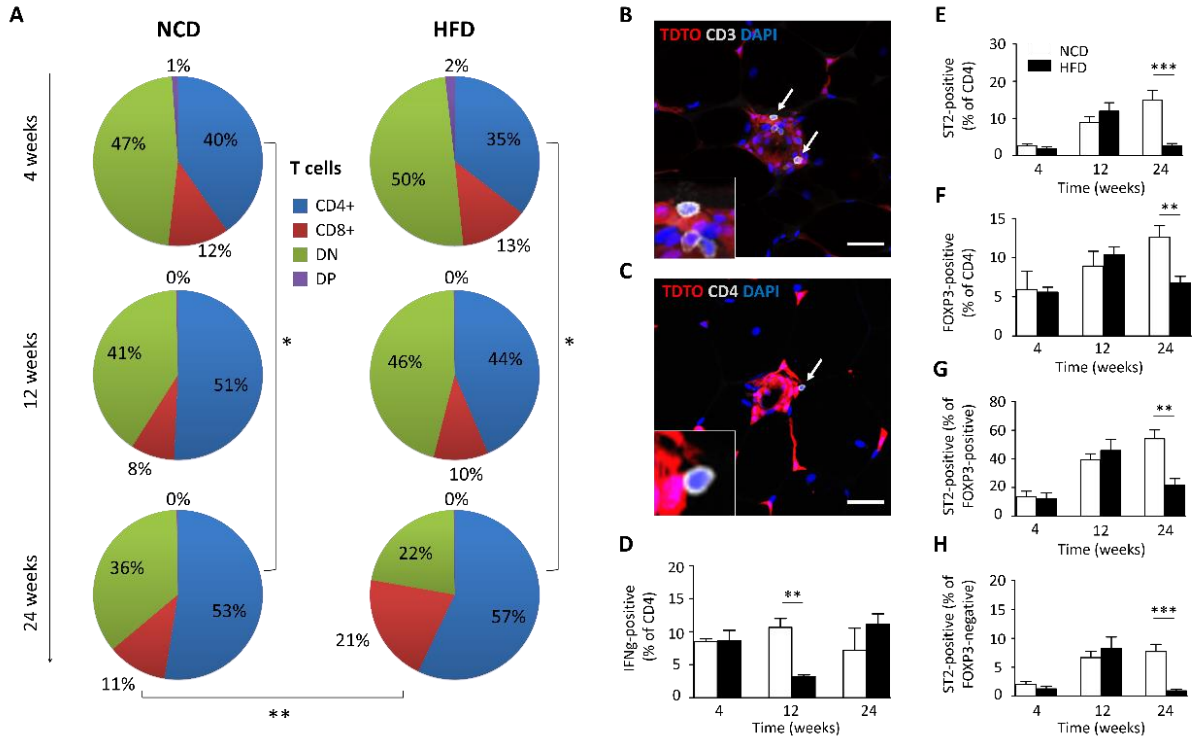



**Figure 1: Immuno-profiling of adipose tissue lymphocytes (ATLs)**

A, Flow cytometric analysis of CD4 and CD8 expression on ATLs in lean (n=4-6) and obese (n=4-7) mice on a C57BL6 background at 4, 12 and 24 weeks. CD4-positive, CD8-positive, double-positive (DP) and double-negative (DN) subsets are depicted as proportion of $CD3^{+}CD45^{+}$ cells. Data come from 8 experiments with 2-6 mice per experiment. B and C, VAT of TDTO reporter mice was stained for CD3 (B; n=3) and CD4 (C; n=3) and DAPI. TDTO is expressed in macrophages and fluoresces red. In representative images of CLS, CD3 and CD4 fluorescence is shown in grey. Data is representative of 2 experiments with 3 mice per experiment. D-H, Flow cytometric immuno-profiling for expression of IFNg, ST2 and FOXP3 among CD4-positive ATLs of mice fed either a NCD (white; n=5-10, cumulative data from >5 independent experiments) or a HFD (black; n=5-10; cumulative data from >5 independent experiments) for 4, 12 or 24 weeks. Data are presented as column bar graphs and as means ± SEM and were tested for statistical significance by Mann-Whitney U-test. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. Scale bar represents 50 µm.

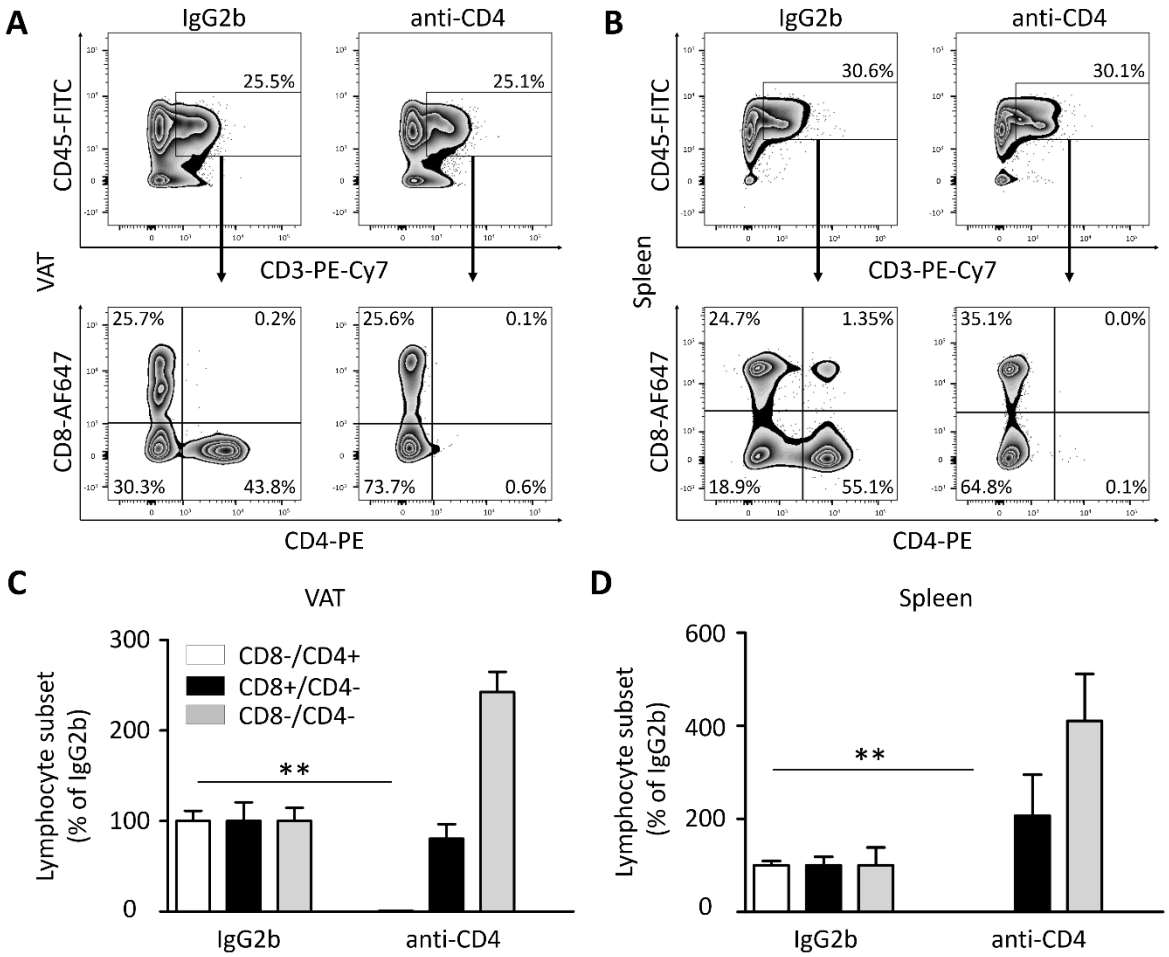



**Figure 2: Flow cytometric analysis of T cell depletion in VAT and spleen**

Efficiency of T cell depletion was assessed using flow cytometry. Cells were stained for the lymphocyte markers CD45, CD3, CD4 and CD8 prior to analysis. A and B, Representative flow cytometry plots for the relative distribution of T cell subsets following depletion of CD4-positive cells or injection of an appropriate isotype control (IgG2b). C and D, CD4-specific antibodies efficiently depleted CD4-positive T cells (white) by ~99% (n=4-6 in both groups; cumulative data from 3 independent experiments). Depletion of CD4-positive T cells was consistent in VAT and spleen. Data are presented as means ± SEM and as column bar graphs and were tested for statistical significance by Mann-Whitney U-test. ** $p < 0.01$.

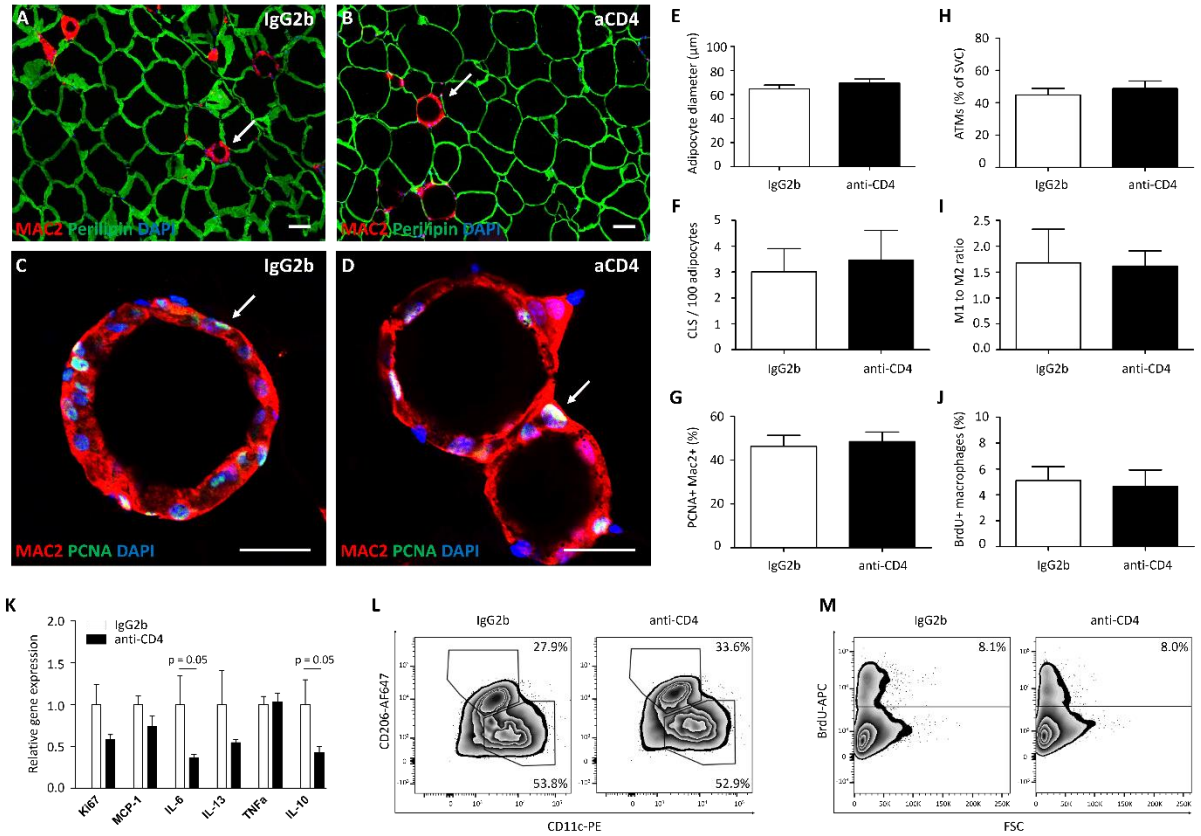



**Figure 3: Effect of 3-day CD4 cell depletion on AT integrity**

A and B, Representative images of immunofluorescence staining for the macrophage marker Mac2 (red), the fat cell marker Perilipin A (green) and DAPI (blue). White arrows show CLS. This immunofluorescence staining was used to quantify fat cell expansion (E) and density of CLS (F) (IgG2b cohort: n=8, anti-CD4 cohort: n=10; cumulative data from 5 independent experiments). H-J, Flow cytometry was used to assess ATM density (H) ATM activation (I) and proliferation (J) (IgG2b cohort: n=4 and anti-CD4 cohort: n=6 in all experiments; each panel represents cumulative data from 3 independent experiments). For flow cytometric analysis of proliferation, AT stromal vascular fraction cells (SVC) were stained for BrdU. C, D and G, For morphological analysis of ATM proliferation, AT was stained for the macrophage marker Mac2 (red), the proliferation marker PCNA (green; white arrows in C and D) and DAPI (blue) (IgG2b cohort: n=8, anti-CD4 cohort: n=10; panel G represents cumulative data from 5 independent experiments, representative images are shown in panel C and D). K, Whole AT gene expression analysis of Ki67 and various ATM proliferation- and ATM activation-associated cytokines was performed in IgG2b (n=3-4) and anti-CD4 (n=5-6) treated mice using quantitative real-time PCR (cumulative data from 3 experiments). Gene of interest mRNA levels were measured in duplicate and normalised to TBP. L and M, Representative flow cytometry plots for M1/M2 and BrdU staining after treatment with IgG2b or CD4 antibody respectively (data analysis is shown in I and J). Data are presented as means ± SEM and as column bar graphs and were tested for statistical significance by Mann-Whitney U-test. All scale bars represent 25 µm.

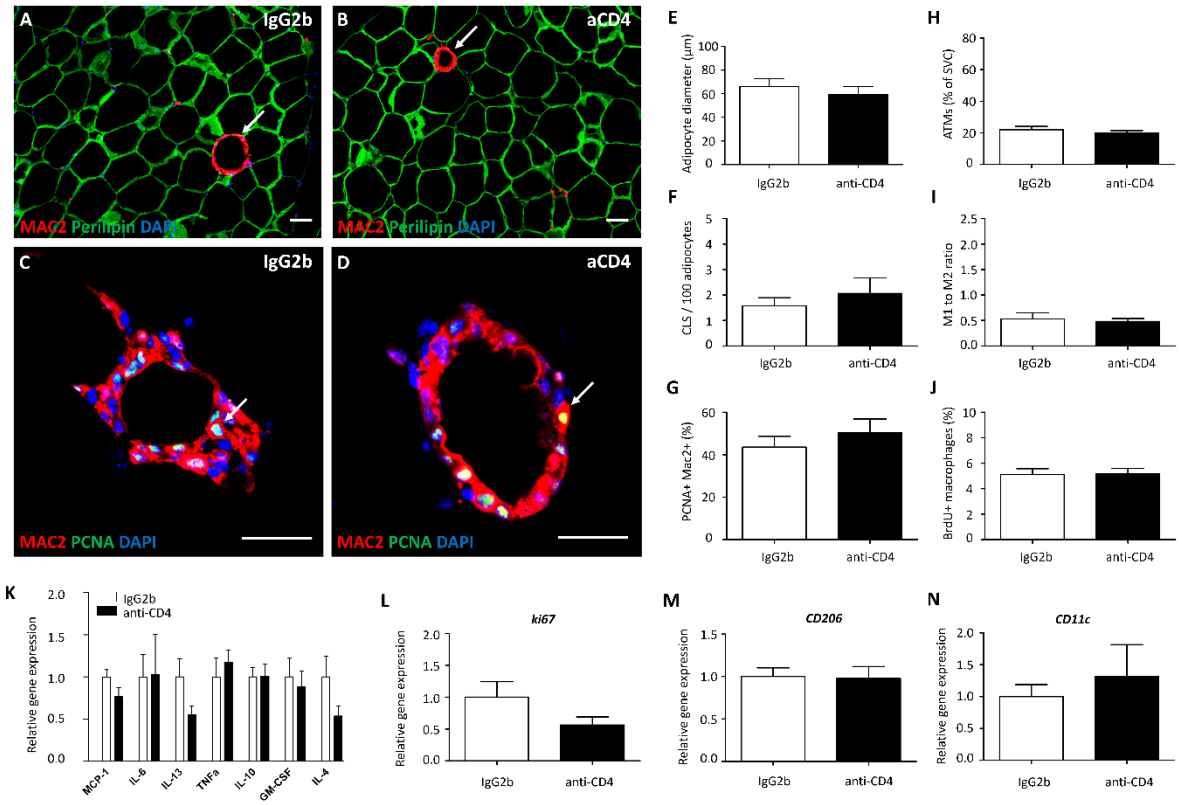



**Figure 4: Effect of 14-day CD4 cell depletion on AT integrity**

A and B, Triple immunofluorescence staining of VAT for the macrophage marker Mac2 (red), the fat cell marker Perilipin A (green) and DAPI (blue) (representative images, data analysis shown in E and F). White arrows show CLS. E and F, Quantification of Perilipin/Mac2 staining as measured by adipocyte diameter (E; n=6 per group; cumulative data from 3 independent experiments) and number of CLS per 100 adipocytes (F; n=6 per group; cumulative data from 3 independent experiments). C and D, Triple immunofluorescence staining of VAT for the macrophage marker Mac2 (red), the proliferation marker PCNA (green; white arrows in C and D) and DAPI (blue) (representative images, data analysis shown in G). G, Analysis of PCNA staining for ATM proliferation (n=6; cumulative data from 3 independent experiments). H-J, Flow cytometric quantification of ATM density (H; both groups n=8), activation (I; both groups n=8) and proliferation (J; both groups n=10) (each panel represents cumulative data from 5 independent experiments). K-N, Data from whole AT gene expression analysis using quantitative real-time PCR (both groups n=6, cumulative data from 3 independent experiments). Gene of interest mRNA levels were measured in duplicate and normalised to TBP. Data are presented as means ± SEM and as column bar graphs and were tested for statistical significance by Mann-Whitney U-test. All scale bars represent 25 µm.

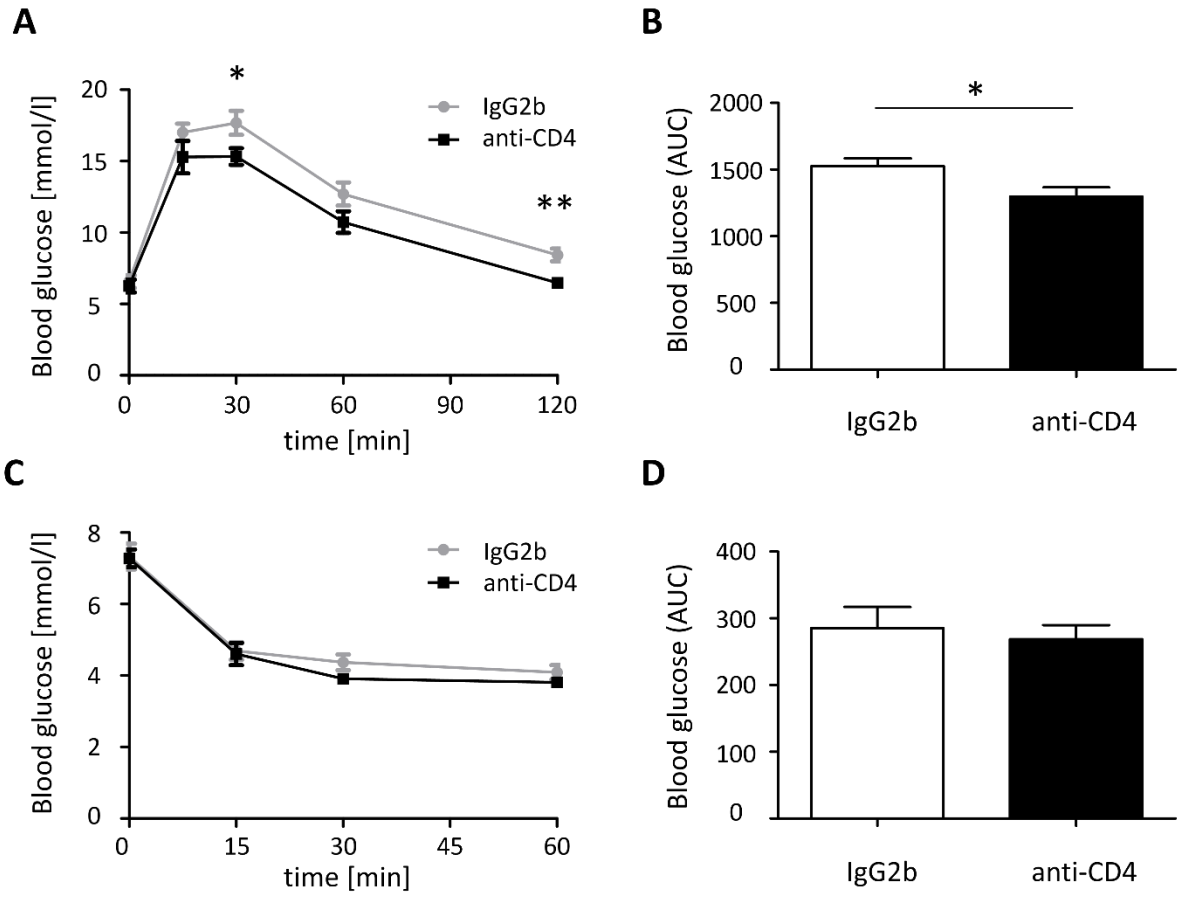



**Figure 5: Impact of 14-day CD4 cell depletion on glucose homeostasis**

A, Glucose tolerance testing (GTT) on mice treated with CD4-depleting or IgG2b control antibodies for 14 days after 24 weeks of HFD-feeding (n=10 per group; cumulative data from 5 independent experiments). B, Area-under-the-curve (AUC) analysis of GTT. C, Insulin tolerance testing (ITT) on mice treated with CD4-depleting or IgG2b control antibodies for 14 days after 24 weeks of HFD-feeding (n=10 per group; cumulative data from 5 independent experiments). D, Area-under-the-curve analysis of ITT. Data are presented as means ± SEM and were tested for statistical significance by unpaired t-testing, * $p < 0.05$. ** $p < 0.01$.

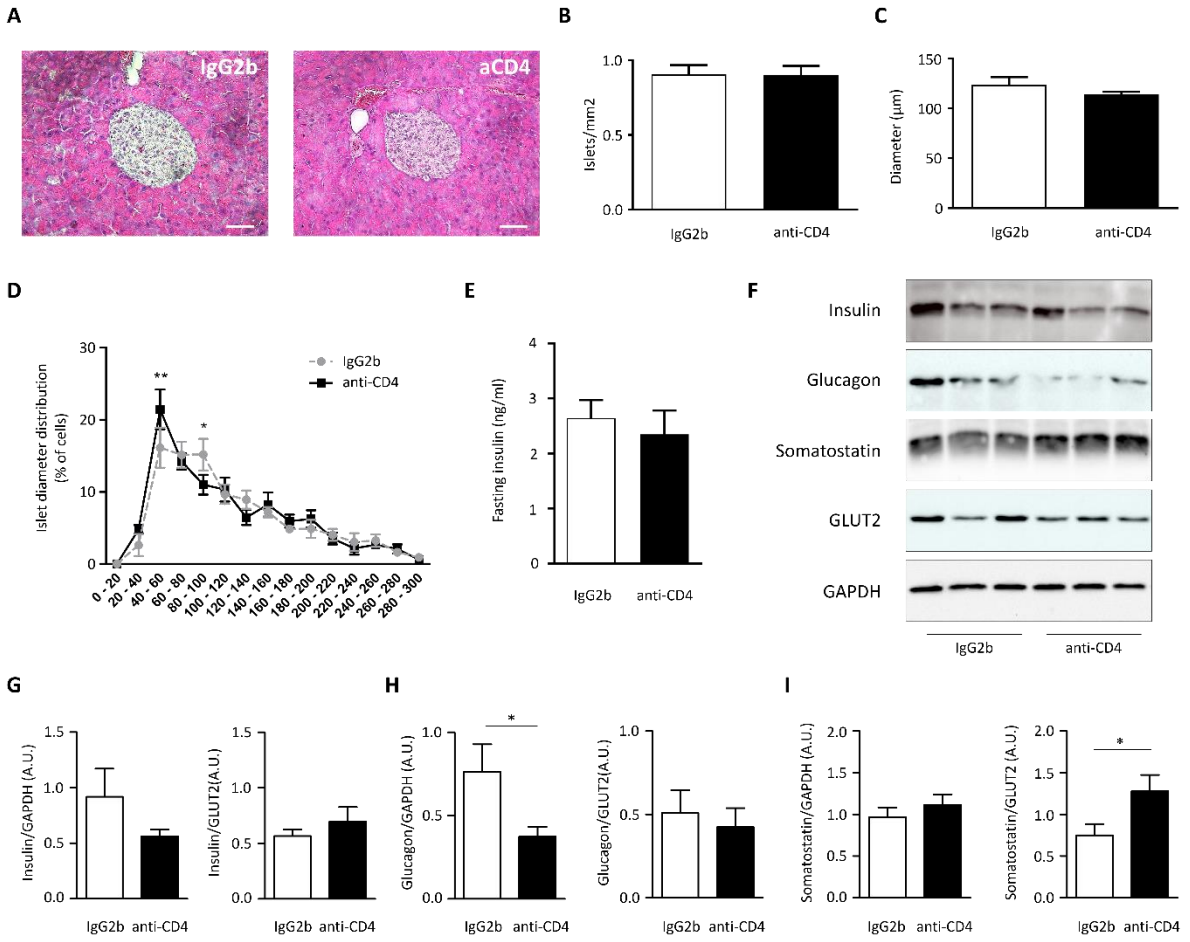
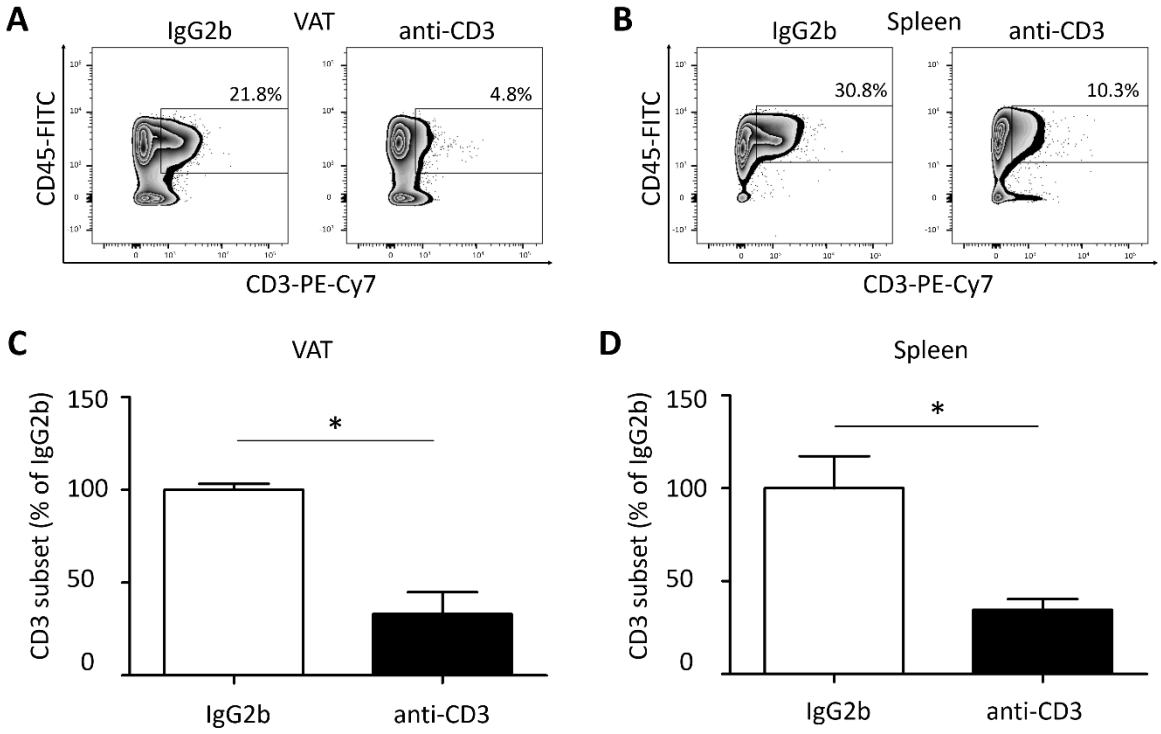



**Figure 6: Impact of 14-day CD4 depletion on pancreatic tissue integrity and secretory function**

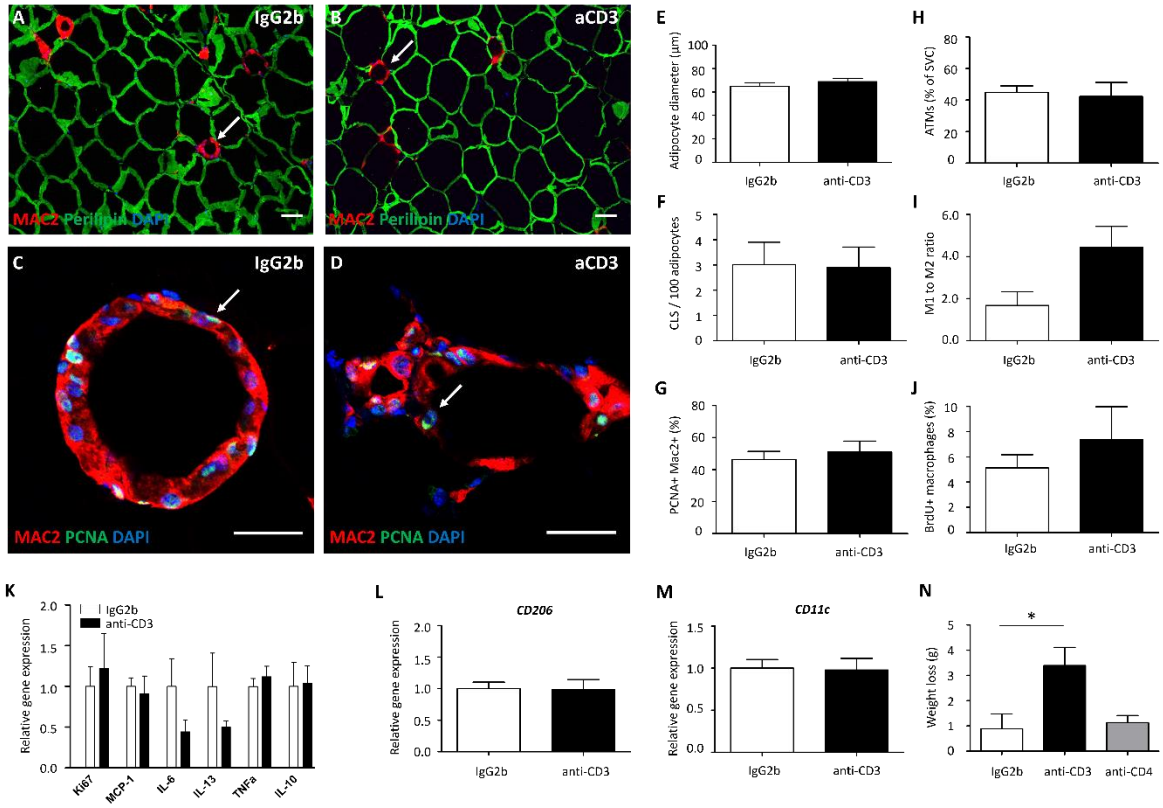
A, Hematoxylin and eosin staining of pancreatic tissue (representative images of islets, data analysis shown in B and C). B and C, Morphometric analysis of pancreatic tissue as measured by number of islets per $mm^2$ pancreatic tissue (B; n=6 per group; cumulative data from 3 independent experiments) and islet diameter (C; n=6 per group; cumulative data from 3 independent experiments). D, Frequency distribution analysis of islet size (shown as histogram with 20 µm bin width (n=6 per group; cumulative data from 3 independent experiments). E, Fasting plasma insulin in mice treated with either CD4-depleting or control antibody was measured using enzyme-linked immunosorbent assay (n=8 per group; cumulative data from 4 independent experiments). F, Representative Western blots of insulin, somatostatin, glucagon, GLUT2 and GAPDH following depletion of CD4-positive cells or injection of an appropriate isotype control. Uncropped Western blots can be found in Suppl. Fig. 4. G-I, The abundance of insulin (G; both groups n=8), glucagon (H; both groups n=8) and somatostatin (I; both groups n=8) is normalised to both GAPDH to account for whole pancreatic cell mass and GLUT2 to account for islet cell mass (panel G, H and I represent cumulative data from 4 independent experiments with 4 mice per experiment). Data are presented as means ± SEM and as column bar graphs and were tested for statistical significance by unpaired t-testing, * $p < 0.05$. ** $p < 0.01$. Scale bar represents 50 µm.

**Supporting information**



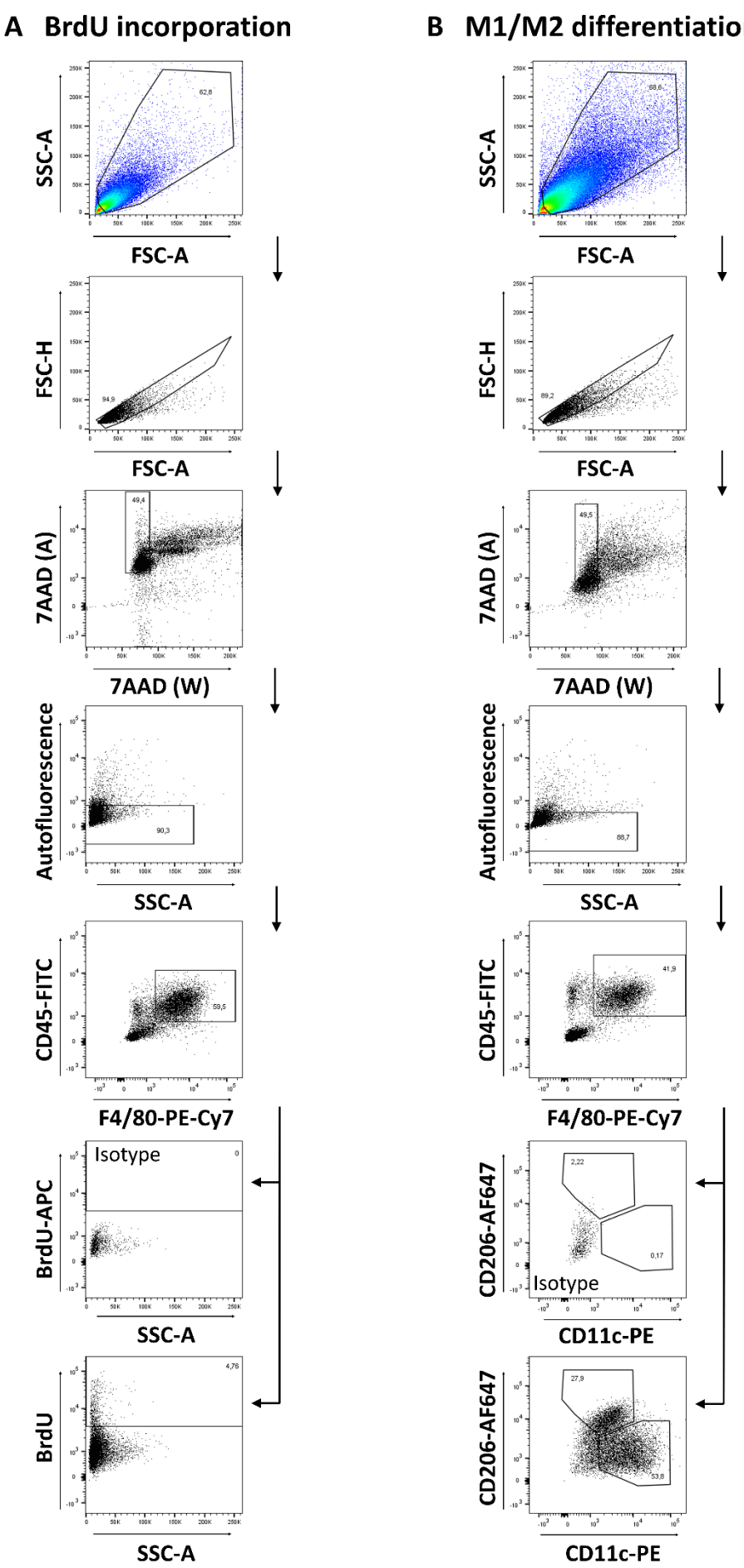

**Supporting Information Figure 1: Flow cytometric validation of CD3 depletion**

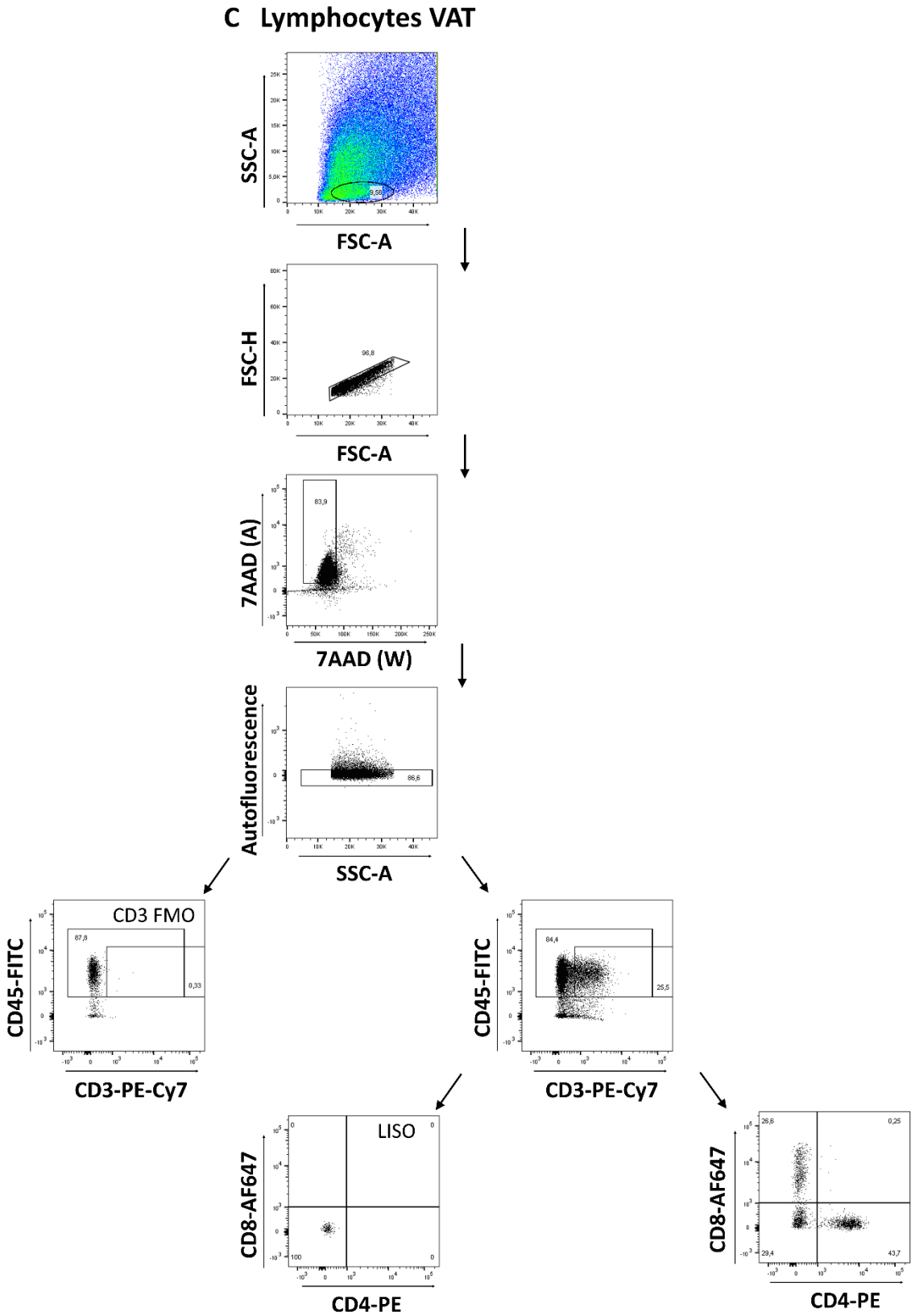
Flow cytometry was used to calculate the efficiency of CD3 depletion in VAT and spleen. Cells were stained for the lymphocyte markers CD45 and CD3 prior to analysis. A and B, Representative flow cytometry plots showing the population of CD3-positive T cells following injection of a CD3-depleting antibody or an appropriate isotype control. C and D, CD3-specific antibodies depleted CD3-positive T cells by ~66% in both VAT and splenic tissue (n=4-5 in both group; 3 independent experiments each). Data are presented as means ± SEM and as column bar graphs and were tested for statistical significance by Mann-Whitney U-test. * $p < 0.05$.

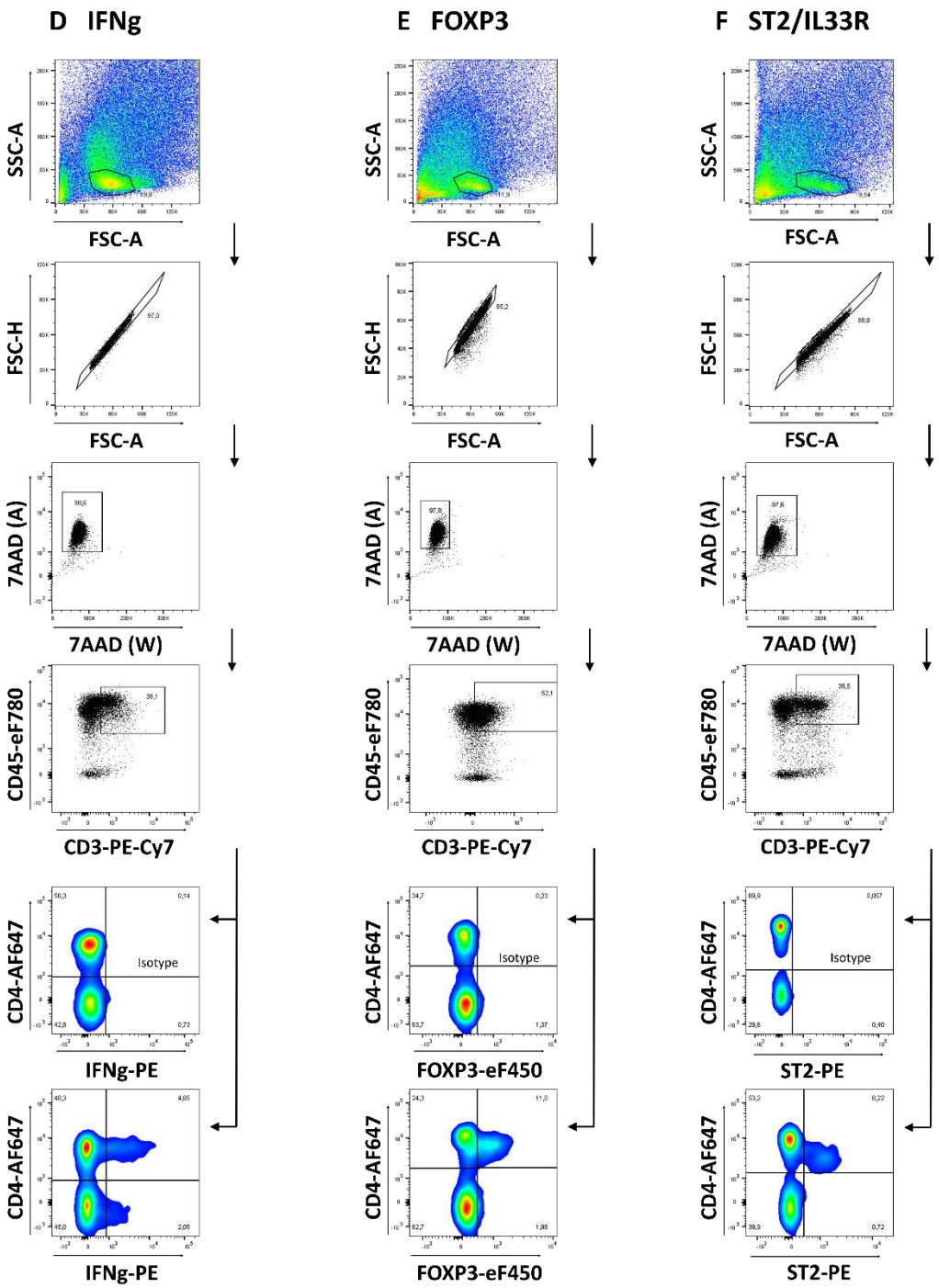



**Supporting Information Figure 2: Effect of 3-day CD3 depletion on AT inflammation**

A and B, Representative images of triple immunofluorescence staining for the macrophage marker Mac2 (red), the fat cell marker Perilipin A (green) and DAPI (blue). Staining was used for morphometric analysis of VAT following injection of CD3-depleting antibody or an isotype control antibody (data shown in E and F). White arrows show CLS. C and D, Representative images of triple immunofluorescence staining for the macrophage marker Mac2 (red), the proliferation marker PCNA (green; white arrows in C and D) and DAPI (blue). Staining was used for quantification of ATM proliferation and activation. E and F, Morphometric analysis of AT as measured by adipocyte diameter (E; n=8 per group; 5 independent experiments) and number of CLS per 100 adipocytes (F; n=8 per group; 5 independent experiments). G, Analysis of PCNA staining for ATM proliferation (IgG2b cohort: n=4, anti-CD3 cohort: n=5; 3 independent experiments). H-J, Flow cytometric quantification of ATM density (H; IgG2b cohort: n=4, anti-CD3 cohort: n=5), activation (I; IgG2b cohort: n=4, anti-CD3 cohort: n=5) and proliferation (J; IgG2b cohort: n=4, anti-CD3 cohort: n=5) (3 independent experiments each). K-M, Data from whole AT gene expression analysis using quantitative real-time PCR (IgG2b cohort: n=3-4, anti-CD3 cohort: n=4-5 for each gene of interest; 3 independent experiments each). Gene of interest mRNA levels were measured in duplicate and normalised to TBP. N, Weight loss following either CD3 or CD4 cell depletion or injection of an appropriate isotype control (IgG2b cohort: n=8, anti-CD3 cohort: n=9, anti-CD4 cohort: n=10; 5 independent experiments). Data are presented as means ± SEM and as column bar graphs and were tested for statistical significance by Mann-Whitney U-test. Scale bar represents 25 µm.

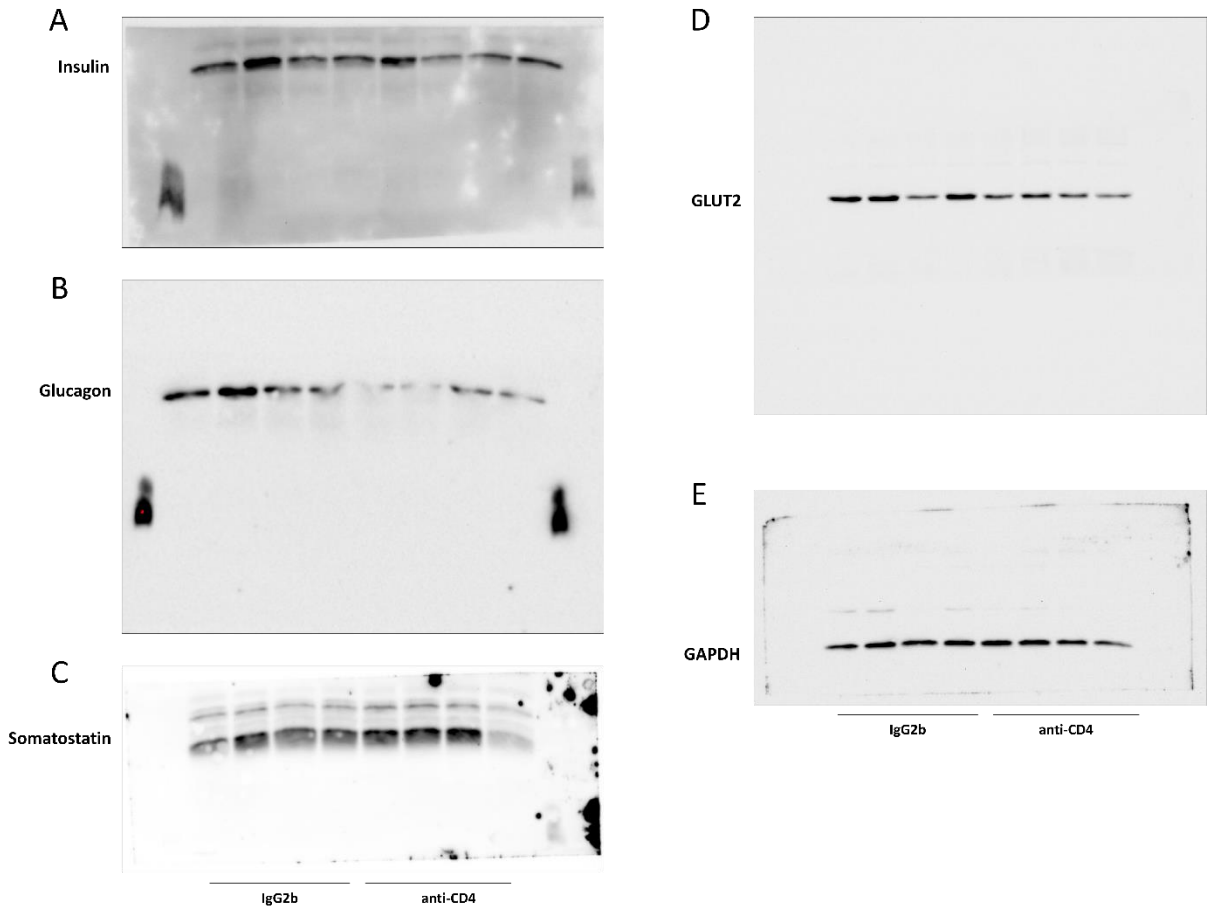

A BrdU incorporation
B M1/M2 differentiation
SSC-A
FSC-A
62,8
68,6
FSC-H
FSC-A
94,9
89,2
7AAD (A)
7AAD (W)
49,4
49,5
Autofluorescence
SSC-A
90,3
88,7
CD45-FITC
F4/80-PE-Cy7
59,5
41,9
BrdU-APC
SSC-A
Isotype
0
CD206-AF647
CD11c-PE
2,22
0,17
Isotype
BrdU
SSC-A
4,76
CD206-AF647
CD11c-PE
27,9
53,8


C Lymphocytes VAT
SSC-A
FSC-A
FSC-H
FSC-A
7AAD (A)
7AAD (W)
Autofluorescence
SSC-A
CD3 FMO
CD45-FITC
CD3-PE-Cy7
CD45-FITC
CD3-PE-Cy7
LISO
CD8-AF647
CD4-PE
CD8-AF647
CD4-PE




**Supporting Information Figure 3: Gating strategy**

A-E, Gating strategies are provided for BrdU incorporation of ATMs (A), M1/M2 differentiation (B), distribution of T cell subsets (C) and expression of IFNg (D), FOXP3 (E) and ST2 (F) on T cells present in VAT.




**Supporting Information Figure 4: Western blot**

A-E, Uncropped Western blots are provided for Insulin (A), Glucagon (B), Somatostatin (C), GLUT 2 (D) and GAPDH (E).

## References


Alegre, M.; Vandenabeele, P.; Flamand, V.; Moser, M.; Leo, O.; Abramowicz, D. et al. (1990) Hypothermia and hypoglycemia induced by anti-CD3 monoclonal antibody in mice: role of tumor necrosis factor. In : European journal of immunology, vol. 20, n° 3, p. 707–710.

Amano, Shinya U.; Cohen, Jessica L.; Vangala, Pranitha; Tencerova, Michaela; Nicoloro, Sarah M.; Yawe, Joseph C. et al. (2014) Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. In : Cell metabolism, vol. 19, n° 1, p. 162–171.

Bagger, Jonatan I.; Knop, Filip K.; Lund, Asger; Holst, Jens J.; Vilsbøll, Tina (2014) Glucagon responses to increasing oral loads of glucose and corresponding isoglycaemic intravenous glucose infusions in patients with type 2 diabetes and healthy individuals. In : Diabetologia, vol. 57, n° 8, p. 1720–1725.

Bapat, Sagar P.; Myoung Suh, Jae; Fang, Sungsoon; Liu, Sihao; Zhang, Yang; Cheng, Albert et al. (2015) Depletion of fat-resident Treg cells prevents age-associated insulin resistance. In : Nature, vol. 528, n° 7580, p. 137–141.

Bourlier, V.; Zakaroff-Girard, A.; Miranville, A.; Barros, S. de; Maumus, M.; Sengenes, C. et al. (2008) Remodeling phenotype of human subcutaneous adipose tissue macrophages. In : Circulation, vol. 117, n° 6, p. 806–815.

Braune, Julia; Weyer, Ulrike; Hobusch, Constance; Mauer, Jan; Brüning, Jens C.; Bechmann, Ingo; Gericke, Martin (2017) IL-6 Regulates M2 Polarization and Local Proliferation of Adipose Tissue Macrophages in Obesity. In : Journal of immunology (Baltimore, Md. : 1950), vol. 198, n° 7, p. 2927–2934.

Stephen A. Bustin, éd. (2004) A - Z of quantitative PCR. La Jolla, Calif. : Internat. Univ. Line (IUL biotechnology series, 5).

Cho, Kae Won; Morris, David L.; Lumeng, Carey N. (2014) Flow cytometry analyses of adipose tissue macrophages. In : Methods in enzymology, vol. 537, p. 297–314.

Cinti, Saverio; Mitchell, Grant; Barbatelli, Giorgio; Murano, Incoronata; Ceresi, Enzo; Faloia, Emanuela et al. (2005) Adipocyte death defines macrophage localization and function in

adipose tissue of obese mice and humans. In : Journal of lipid research, vol. 46, n° 11, p. 2347–2355.

Cipolletta, Daniela; Cohen, Paul; Spiegelman, Bruce M.; Benoist, Christophe; Mathis, Diane (2015) Appearance and disappearance of the mRNA signature characteristic of Treg cells in visceral adipose tissue. Age, diet, and PPARγ effects. In : Proceedings of the National Academy of Sciences of the United States of America, vol. 112, n° 2, p. 482–487.

Cipolletta, Daniela; Feuerer, Markus; Li, Amy; Kamei, Nozomu; Lee, Jongsoon; Shoelson, Steven E. et al. (2012) PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. In : Nature, vol. 486, n° 7404, p. 549–553.

Cossarizza, Andrea; Chang, Hyun-Dong; Radbruch, Andreas; Akdis, Mübeccel; Andrä, Immanuel; Annunziato, Francesco et al. (2017) Guidelines for the use of flow cytometry and cell sorting in immunological studies. In : European journal of immunology, vol. 47, n° 10, p. 1584–1797.

Deng, Tuo; Liu, Joey; Deng, Yanru; Minze, Laurie; Xiao, Xiang; Wright, Valerie et al. (2017) Adipocyte adaptive immunity mediates diet-induced adipose inflammation and insulin resistance by decreasing adipose Treg cells. In : Nature Communications, vol. 8, n° 1, p. 1–11.

Deng, Tuo; Lyon, Christopher J.; Minze, Laurie J.; Lin, Jianxin; Zou, Jia; Liu, Joey Z. et al. (2013) Class II major histocompatibility complex plays an essential role in obesity-induced adipose inflammation. In : Cell metabolism, vol. 17, n° 3, p. 411–422.

Economic Costs of Diabetes in the U.S. in 2017 (2018). In : Diabetes care, vol. 41, n° 5, p. 917–928.

Eller, Kathrin; Kirsch, Alexander; Wolf, Anna M.; Sopper, Sieghart; Tagwerker, Andrea; Stanzl, Ursula et al. (2011) Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. In : Diabetes, vol. 60, n° 11, p. 2954–2962.

Ferran, C.; Dy, M.; Sheehan, K.; Merite, S.; Schreiber, R.; Landais, P. et al. (1991) Inter-mouse strain differences in the in vivo anti-CD3 induced cytokine release. In : Clinical and experimental immunology, vol. 86, n° 3, p. 537–543.

Ferran, C.; Sheehan, K.; Dy, M.; Schreiber, R.; Merite, S.; Landais, P. et al. (1990) Cytokine-related syndrome following injection of anti-CD3 monoclonal antibody: further evidence for transient in vivo T cell activation. In : European journal of immunology, vol. 20, n° 3, p. 509–515.

Feuerer, Markus; Herrero, Laura; Cipolletta, Daniela; Naaz, Afia; Wong, Jamie; Nayer, Ali et al. (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. In : Nature medicine, vol. 15, n° 8, p. 930–939.

Gastaldelli, Amalia; Miyazaki, Yoshinori; Pettiti, Maura; Matsuda, Masafumi; Mahankali, Srihanth; Santini, Eleonora et al. (2002) Metabolic effects of visceral fat accumulation in type 2 diabetes. In : The Journal of clinical endocrinology and metabolism, vol. 87, n° 11, p. 5098–5103.

Gericke, Martin; Weyer, Ulrike; Braune, Julia; Bechmann, Ingo; Eilers, Jens (2015) A method for long-term live imaging of tissue macrophages in adipose tissue explants. In : American journal of physiology. Endocrinology and metabolism, vol. 308, n° 11, E1023-33.

Gromada, Jesper; Chabosseau, Pauline; Rutter, Guy A. (2018) The α-cell in diabetes mellitus. In : Nature Reviews Endocrinology, vol. 14, n° 12, p. 694–704.

Haase, Julia; Weyer, Ulrike; Immig, Kerstin; Klöting, Nora; Blüher, Matthias; Eilers, Jens et al. (2014) Local proliferation of macrophages in adipose tissue during obesity-induced inflammation. In : Diabetologia, vol. 57, n° 3, p. 562–571.

Hædersdal, Sofie; Lund, Asger; Knop, Filip K.; Vilsbøll, Tina (2018) The Role of Glucagon in the Pathophysiology and Treatment of Type 2 Diabetes. In : Mayo Clinic Proceedings, vol. 93, n° 2, p. 217–239.

Han, Jonathan M.; Wu, Dan; Denroche, Heather C.; Yao, Yu; Verchere, C. Bruce; Levings, Megan K. (2015a) IL-33 Reverses an Obesity-Induced Deficit in Visceral Adipose Tissue ST2+ T Regulatory Cells and Ameliorates Adipose Tissue Inflammation and Insulin Resistance. In : Journal of immunology (Baltimore, Md. : 1950), vol. 194, n° 10, p. 4777–4783.

Han, Jonathan M.; Wu, Dan; Denroche, Heather C.; Yao, Yu; Verchere, C. Bruce; Levings, Megan K. (2015b) IL-33 Reverses an Obesity-Induced Deficit in Visceral Adipose Tissue ST2+

T Regulatory Cells and Ameliorates Adipose Tissue Inflammation and Insulin Resistance. In : Journal of immunology (Baltimore, Md. : 1950), vol. 194, n° 10, p. 4777–4783.

James, W. P. T. (2008) WHO recognition of the global obesity epidemic. In : International journal of obesity (2005), 32 Suppl 7, S120-6.

Kellard, Joely A.; Rorsman, Nils J. G.; Hill, Thomas G.; Armour, Sarah L.; van de Bunt, Martijn; Rorsman, Patrik et al. (2020) Reduced somatostatin signalling leads to hypersecretion of glucagon in mice fed a high-fat diet. In : Molecular metabolism, vol. 40, p. 101021.

Kintscher, Ulrich; Hartge, Martin; Hess, Katharina; Foryst-Ludwig, Anna; Clemenz, Markus; Wabitsch, Martin et al. (2008) T-lymphocyte infiltration in visceral adipose tissue. A primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. In : Arteriosclerosis, thrombosis, and vascular biology, vol. 28, n° 7, p. 1304–1310.

Klöting, Nora; Fasshauer, Mathias; Dietrich, Arne; Kovacs, Peter; Schön, Michael R.; Kern, Matthias et al. (2010) Insulin-sensitive obesity. In : American journal of physiology. Endocrinology and metabolism, vol. 299, n° 3, E506-15.

Knop, Filip K.; Aaboe, K.; Vilsbøll, T.; Vølund, A.; Holst, J. J.; Krarup, T.; Madsbad, S. (2012) Impaired incretin effect and fasting hyperglucagonaemia characterizing type 2 diabetic subjects are early signs of dysmetabolism in obesity. In : Diabetes, obesity & metabolism, vol. 14, n° 6, p. 500–510.

Li, Chaoran; DiSpirito, Joanna R.; Zemmour, David; Spallanzani, Raul German; Kuswanto, Wilson; Benoist, Christophe; Mathis, Diane (2018) TCR Transgenic Mice Reveal Stepwise, Multi-site Acquisition of the Distinctive Fat-Treg Phenotype. In : Cell, vol. 174, n° 2, 285-299.e12.

Liu, Jian; Divoux, Adeline; Sun, Jiusong; Zhang, Jie; Clément, Karine; Glickman, Jonathan N. et al. (2009) Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. In : Nature medicine, vol. 15, n° 8, p. 940–945.

Lumeng, Carey N.; Bodzin, Jennifer L.; Saltiel, Alan R. (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. In : The Journal of clinical investigation, vol. 117, n° 1, p. 175–184.

Martinez, Fernando O.; Gordon, Siamon (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. In : F1000prime reports, vol. 6, p. 13.

Merino, Beatriz; Alonso-Magdalena, Paloma; Lluesma, Mónica; Ñeco, Patricia; Gonzalez, Alejandro; Marroquí, Laura et al. (2015) Pancreatic alpha-cells from female mice undergo morphofunctional changes during compensatory adaptations of the endocrine pancreas to diet-induced obesity. In : Scientific Reports, vol. 5, n° 1, p. 11622.

Miller, Ashley M.; Asquith, Darren L.; Hueber, Axel J.; Anderson, Lesley A.; Holmes, William M.; McKenzie, Andrew N. et al. (2010) Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. In : Circulation research, vol. 107, n° 5, p. 650–658.

Müller, W. A.; Faloona, G. R.; Aguilar-Parada, E.; Unger, R. H. (1970) Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion. In : The New England journal of medicine, vol. 283, n° 3, p. 109–115.

Newgard, Christopher B.; An, Jie; Bain, James R.; Muehlbauer, Michael J.; Stevens, Robert D.; Lien, Lillian F. et al. (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. In : Cell metabolism, vol. 9, n° 4, p. 311–326.

Nishimura, Satoshi; Manabe, Ichiro; Nagasaki, Mika; Eto, Koji; Yamashita, Hiroshi; Ohsugi, Mitsuru et al. (2009) CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. In : Nature medicine, vol. 15, n° 8, p. 914–920.

Omar-Hmeadi, Muhmmad; Lund, Per-Eric; Gandasi, Nikhil R.; Tengholm, Anders; Barg, Sebastian (2020) Paracrine control of α-cell glucagon exocytosis is compromised in human type-2 diabetes. In : Nature communications, vol. 11, n° 1, p. 1896.

O'Rourke, R. W.; Metcalf, M. D.; White, A. E.; Madala, A.; Winters, B. R.; Maizlin, I. I. et al. (2009) Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-gamma in inflammation in human adipose tissue. In : International journal of obesity (2005), vol. 33, n° 9, p. 978–990.

Orthgiess, Johannes; Gericke, Martin; Immig, Kerstin; Schulz, Angela; Hirrlinger, Johannes; Bechmann, Ingo; Eilers, Jens (2016) Neurons exhibit Lyz2 promoter activity in vivo:

Implications for using LysM-Cre mice in myeloid cell research. In : European journal of immunology, vol. 46, n° 6, p. 1529–1532.

Osborn, Olivia; Olefsky, Jerrold M. (2012) The cellular and signaling networks linking the immune system and metabolism in disease. In : Nature medicine, vol. 18, n° 3, p. 363–374.

Roat, Regan; Rao, Vandana; Doliba, Nicolai M.; Matschinsky, Franz M.; Tobias, John W.; Garcia, Eden et al. (2014) Alterations of pancreatic islet structure, metabolism and gene expression in diet-induced obese C57BL/6J mice. In : PloS one, vol. 9, n° 2, e86815.

Sasmono, R. Tedjo; Oceandy, Delvac; Pollard, Jeffrey W.; Tong, Wei; Pavli, Paul; Wainwright, Brandon J. et al. (2003) A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. In : Blood, vol. 101, n° 3, p. 1155–1163.

Shirakawa, Kohsuke; Yan, Xiaoxiang; Shinmura, Ken; Endo, Jin; Kataoka, Masaharu; Katsumata, Yoshinori et al. (2016) Obesity accelerates T cell senescence in murine visceral adipose tissue. In : The Journal of clinical investigation, vol. 126, n° 12, p. 4626–4639.

Stern, Jennifer H.; Smith, Gordon I.; Chen, Shiuwei; Unger, Roger H.; Klein, Samuel; Scherer, Philipp E. (2019) Obesity dysregulates fasting-induced changes in glucagon secretion. In : Journal of Endocrinology, vol. 243, n° 2, p. 149–160.

Strissel, Katherine J.; DeFuria, Jason; Shaul, Merav E.; Bennett, Grace; Greenberg, Andrew S.; Obin, Martin S. (2010) T-cell recruitment and Th1 polarization in adipose tissue during diet-induced obesity in C57BL/6 mice. In : Obesity (Silver Spring, Md.), vol. 18, n° 10, p. 1918–1925.

Strissel, Katherine J.; Stancheva, Zlatina; Miyoshi, Hideaki; Perfield, James W.; DeFuria, Jason; Jick, Zoe et al. (2007) Adipocyte death, adipose tissue remodeling, and obesity complications. In : Diabetes, vol. 56, n° 12, p. 2910–2918.

Strowski, M. Z.; Parmar, R. M.; Blake, A. D.; Schaeffer, J. M. (2000) Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice. In : Endocrinology, vol. 141, n° 1, p. 111–117.

Talukdar, Saswata; Oh, Da Young; Bandyopadhyay, Gautam; Li, Dongmei; Xu, Jianfeng; McNelis, Joanne et al. (2012) Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. In : Nature medicine, vol. 18, n° 9, p. 1407–1412.

Tardelli, Matteo; Zeyda, Karina; Moreno-Viedma, Veronica; Wanko, Bettina; Grün, Nicole G.; Staffler, Günther et al. (2016) Osteopontin is a key player for local adipose tissue macrophage proliferation in obesity. In : Molecular metabolism, vol. 5, n° 11, p. 1131–1137.

Vasanthakumar, Ajithkumar; Moro, Kazuyo; Xin, Annie; Liao, Yang; Gloury, Renee; Kawamoto, Shimpei et al. (2015) The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. In : Nature immunology, vol. 16, n° 3, p. 276–285.

Vossen, A. C.; Knulst, A. C.; Tibbe, G. J.; van Oudenaren, A.; Baert, M. R.; Benner, R.; Savelkoul, H. F. (1994) Suppression of skin allograft rejection in mice by anti-CD3 monoclonal antibodies without cytokine-related side-effects. In : Transplantation, vol. 58, n° 2, p. 257–261.

Vossen, A. C.; Tibbe, G. J.; Kroos, M. J.; van de Winkel, J. G.; Benner, R.; Savelkoul, H. F. (1995) Fc receptor binding of anti-CD3 monoclonal antibodies is not essential for immunosuppression, but triggers cytokine-related side effects. In : European journal of immunology, vol. 25, n° 6, p. 1492–1496.

Wagner, Róbert; Hakaste, Liisa H.; Ahlqvist, Emma; Heni, Martin; Machann, Jürgen; Schick, Fritz et al. (2017) Nonsuppressed Glucagon After Glucose Challenge as a Potential Predictor for Glucose Tolerance. In : Diabetes, vol. 66, n° 5, p. 1373–1379.

Weisberg, Stuart P.; McCann, Daniel; Desai, Manisha; Rosenbaum, Michael; Leibel, Rudolph L.; Ferrante, Anthony W. (2003) Obesity is associated with macrophage accumulation in adipose tissue. In : The Journal of clinical investigation, vol. 112, n° 12, p. 1796–1808.

Winer, Daniel A.; Winer, Shawn; Shen, Lei; Wadia, Persis P.; Yantha, Jason; Paltser, Geoffrey et al. (2011) B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. In : Nature medicine, vol. 17, n° 5, p. 610–617.

Winer, Shawn; Chan, Yin; Paltser, Geoffrey; Truong, Dorothy; Tsui, Hubert; Bahrami, Jasmine et al. (2009) Normalization of obesity-associated insulin resistance through immunotherapy. In : Nature medicine, vol. 15, n° 8, p. 921–929.

Zamarron, Brian F.; Mergian, Taleen A.; Cho, Kae Won; Martinez-Santibanez, Gabriel; Luan, Danny; Singer, Kanakadurga et al. (2017) Macrophage Proliferation Sustains Adipose Tissue Inflammation in Formerly Obese Mice. In : Diabetes, vol. 66, n° 2, p. 392–406.

Zeng, Qin; Sun, Xiaoxiao; Xiao, Liuling; Xie, Zhiguo; Bettini, Maria; Deng, Tuo (2018) A Unique Population: Adipose-Resident Regulatory T Cells. In : Frontiers in immunology, vol. 9, p. 2075.

Zheng, C.; Yang, Q.; Cao, J.; Xie, N.; Liu, K.; Shou, P. et al. (2016) Local proliferation initiates macrophage accumulation in adipose tissue during obesity. In : Cell death & disease, vol. 7, e2167.

Zibolka, Juliane; Wolf, Anja; Rieger, Lisa; Rothgänger, Candy; Jörns, Anne; Lutz, Beat et al. (2020) Influence of Cannabinoid Receptor Deficiency on Parameters Involved in Blood Glucose Regulation in Mice. In : International journal of molecular sciences, vol. 21, n° 9.

# 5 Zusammenfassung

Dissertation zur Erlangung des akademischen Grades Dr. med. an der Medizinischen Fakultät der Universität Leipzig

| | |
|---|---|
| Titel: | **Einfluss CD4-positiver Lymphozyten auf die adipositasassoziierte Inflammation im murinen Fettgewebe** |
| eingereicht von: | Georg Friedrich Brinker<br>geb. am 13.11.1993 in Kiel |
| angefertigt am: | Institut für Anatomie<br>Medizinische Fakultät<br>Universität Leipzig |
| Betreut von: | Herrn Prof. Dr. med. Martin Gericke<br>Herrn Prof. Dr. med. Ingo Bechmann |
| eingereicht im: | Mai 2021 |

Adipositas ist im humanen wie im murinen Fettgewebe mit einer chronischen Entzündungsreaktion vergesellschaftet. Diese Entzündungsreaktion ist charakterisiert durch einen Anstieg von Immunzellen, insbesondere der Makrophagen. Die Akkumulation von Makrophagen im Fettgewebe wird einerseits durch Rekrutierung monozytärer Vorläuferzellen aus dem Blut und andererseits durch lokale Proliferation geweberesidenter Makrophagen gewährleistet. Diese Proliferation scheint von TH2-assoziierten Zytokinen beeinflusst zu werden, weshalb wir die Hypothese aufstellten, dass das adaptive Immunsystem, insbesondere die CD4-positiven T-Lymphozyten, einen Einfluss auf die Makrophagenproliferation im Fettgewebe haben.

T-Lymphozyten spielen eine wichtige Rolle in der adipositasinduzierten Fettgewebeinflammation und ihr Einfluss auf Parameter der Fettgewebeinflammation und des Glukosestoffwechsels wurde in vielen Studien diskutiert. Art und Ausmaß dieses Einflusses sind jedoch nicht abschließend geklärt. Eine wegweisende Studie konnte demonstrieren, dass die CD8-positiven T-Zellen sowohl die Makrophagenaktivierung, als auch die Glukosehomöostase beeinflussen. Ein ähnlicher Effekt wurde auch für CD4-positive Lymphozyten postuliert. Diese These ist allerdings nie in einem *In-vivo*-Depletionsmodell validiert worden.

Um die Rolle der CD4-positiven Lymphozyten im Fettgewebe adipöser Mäuse besser zu verstehen, haben wir daher Mäusen nach 20 Wochen Fettdiät an drei aufeinanderfolgenden Tagen zelldepletierende Antikörper gegen das CD4-Antigen oder aber eine geeignete Immunglobulinkontrolle appliziert. Die Depletion der CD4-Zellen zeigte durchflusszytometrisch eine Effizienz von über 99% und keine adversen Effekte, wie beispielsweise Gewichtsverlust. Wir konzipierten außerdem ein ähnliches Depletionsmodell für CD3-positive Lymphozyten, mussten allerdings feststellen, dass bei einigen Tieren unter CD3-Depletion Peritonitis-ähnliche Reaktionen auftraten, die mit erheblichem Gewichtsverlust einhergingen. Um eine Verzerrung unserer Daten durch den Gewichtsverlust und die Reaktion der Tiere auf den CD3-Antikörper zu vermeiden, haben wir uns bei den weiterführenden Versuchen auf die Analyse der CD4-depletierten Tiere beschränkt.

Zunächst haben wir eruiert, ob sich die CD4-Depletion im Phänotyp des Fettgewebes niederschlägt. Das Auftreten von sogenannten *crown-like structures* (CLS) ist dabei ein geeignetes Maß für den Fortschritt der Fettgewebeinflammation. CLS sind Formationen von Makrophagen um sterbende Fettzellen, die unter hochkalorischer Diät vermehrt auftreten. Wir haben daher in einem ersten Schritt Proben vom epididymalen Fettgewebe mit spezifischen Antikörpern gegen den Makrophagenmarker Mac2 und den Adipozytenmarker Perilipin gefärbt. Es konnten unter CD4-Depletion keine signifikanten Veränderungen in der Dichte der CLS im Fettgewebe festgestellt werden. Die durchflusszytometrische und immunhistochemische Analyse der Proliferation mittels Untersuchung der Einlagerung von BrdU und Färbung des Proliferationsmarkers *proliferating cell nuclear antigen* (PCNA) zeigte keinen Einfluss der CD4-Depletion auf die Selbsterneuerung der Fettgewebemakrophagen. Diese Ergebnisse brachten uns zu der Annahme, dass ein kurzes Depletionsprotokoll und damit wahrscheinlich primär die unmittelbare Zell-Zell-Interaktion zwischen CD4-positiven T-Zellen und Makrophagen offensichtlich keinen erheblichen Einfluss auf die Makrophagenproliferation hat. Allerdings konnten wir in der Genexpressionsanalyse von Fettgewebeproben der CD4-depletierten Tiere und entsprechender Kontrollen einen Trend zur Herunterregulierung proliferationsassoziierter mRNA unter CD4-Depletion beobachten. In einem nächsten Schritt wollten wir daher prüfen, ob eine längere Depletionsdauer durch Veränderungen des Mikromilieus in der Tat die Proliferation und Aktivierung von Fettgewebemakrophagen beeinflusst.

Wir depletierten daher Mäuse nach 20 Wochen Fettdiät über zwei Wochen an jeweils drei Tagen pro Woche mit zelldepletierenden Antikörpern gegen das CD4-Antigen. Allerdings zeigte sich auch in diesem längeren Depletionsprotokoll, trotz der fast absoluten systemischen Depletion von CD4-Zellen, keine Veränderung der Fettgewebeinflammation. Wir stellten allerdings fest, dass die Depletion zu einer signifikanten Verbesserung der Glukosetoleranz führte, ohne jedoch die Insulinsensitivität signifikant zu verbessern. Diese Diskrepanz führte uns zu

der Frage, ob die CD4-Depletion einen Effekt auf das Pankreas habe und in der Tat fanden wir bei unseren weiteren Untersuchungen eine Beeinträchtigung der Glukagon- und Somatostatinachse. Interessanterweise schien die Insulinproduktion jedoch unbeeinträchtigt zu sein.

Im Anschluss an die Lymphozytendepletion wurde außerdem eine Zeitreihe erstellt, mit der das Fettgewebe von Mäusen nach 4, 12 und 24 Wochen Fettdiät auf distinkte, phänotypische Veränderungen innerhalb der T-Zell-Populationen untersucht wurde. Dabei zeigte sich, dass die Zunahme der CD4-Zellen altersabhängig, aber nicht diätabhängig zu sein scheint. Die Population der antiinflammatorischen regulatorischen T-Zellen, charakterisiert durch die Marker ST2 und FoxP3, zeigte eine Abnahme unter hochkalorischer Diät im Vergleich zur Kontrolle. Interessanterweise nahmen in unserer Studie auch die proinflammatorischen IFNg-produzierenden CD4-Zellen unter hochkalorischer Diät zunächst ab, stiegen im Verlauf der Diät jedoch wieder auf das Level der Tiere unter Normaldiät an. Der Einfluss einer hochkalorischen Diät auf die Population der CD4-positiven T-Zellen scheint also weniger ihre Quantität als vielmehr ihre Qualität zu betreffen.

In Zusammenschau konnten wir mit unserer Studie diätabhängige, phänotypische Veränderungen in CD4-positiven T-Zellen demonstrieren und einen Einfluss CD4-positiver Zellen auf die Fettgewebeinflammation, insbesondere die Aktivierung und Proliferation der Fettgewebemakrophagen, ausschließen. Ungeachtet dessen, zeigte sich eine signifikante Verbesserung der Glukosetoleranz nach Depletion CD4-positiver T-Zellen. Diese Beeinflussung der Glukosehomöostase könnte mutmaßlich in einer Dysregulation der endokrinen Funktion des Pankreas oder aber in Veränderungen des Mikromilieus insulinabhängiger Zielorgane begründet sein, die wir mit unserer Studie nicht erfasst haben.

# 6 Erklärung über den wissenschaftlichen Beitrag des Promovenden zu der Publikation

**Publikation:**

**Brinker, G., Froeba, J., Arndt, L., Braune, J., Hobusch, C., Lindhorst, A., Bechmann, I. and Gericke, M.** (2021), CD4+ T cells regulate glucose homeostasis independent of adipose tissue dysfunction in mice. Eur. J. Immunol..

Die Zielsetzung und die grundlegende experimentelle Konzeption dieser Arbeit wurde insbesondere durch Herrn Prof. Martin Gericke erarbeitet. Herr Prof. Martin Gericke übernahm auch die hauptsächliche Betreuung dieser Arbeit und ermöglichte zusammen mit Herrn Prof. Ingo Bechmann ihre Durchführung und Fertigstellung. Herr Georg Brinker übernahm den maßgeblichen Anteil an der Durchführung und Auswertung der experimentellen Arbeit und ebenso die Erstellung des Manuskripts. In die praktisch-experimentelle Arbeit wurde Herr Georg Brinker insbesondere von Frau Constance Hobusch und Frau Julia Braune eingearbeitet. Weitere wichtige Beiträge zu dem vorliegenden Manuskript lieferten die übrigen im Manuskript genannten Ko-Autoren. Aufgrund seines maßgeblichen Anteils an der Entstehung dieser Arbeit ist Georg Brinker alleiniger Erstautor des vorliegenden Manuskripts. Alle Ko-Autoren haben ausdrücklich der Veröffentlichung der Publikation stattgegeben.

Korrespondierender Autor:

_____

Prof. Dr. Martin Gericke

Ausgewählte Koautoren:

_____

Prof. Dr. Ingo Bechmann

_____

M. Sc. Andreas Lindhorst

_____

Constance Hobusch

## 7 Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

| | |
|---|---|
| ..... | ..... |
| Datum | Unterschrift |

# 8 Veröffentlichungen im Rahmen der Arbeit

**Publikation:**

**Brinker, G., Froeba, J., Arndt, L., Braune, J., Hobusch, C., Lindhorst, A., Bechmann, I. and Gericke, M.** (2021), CD4+ T cells regulate glucose homeostasis independent of adipose tissue dysfunction in mice. Eur. J. Immunol..

**Posterpräsentationen:**

Keystone Symposium, The Resolution of Inflammation in Health and Disease, Dublin 2018

Brinker G, Braune J, Hobusch C, Bechmann I, Gericke M

**T-cell depletion does not alter adipose tissue macrophage proliferation**

14th Leipzig Research Festival for Life Sciences, 2018

Brinker G, Braune J, Hobusch C, Bechmann I, Gericke M

**T-cell depletion does not alter adipose tissue macrophage proliferation**

# 9 Danksagung

Mein allergrößter Dank gilt an dieser Stelle Martin Gericke für seine unablässige Unterstützung in Labor- und Lebensfragen, seinen beneidenswerten, unerschütterlichen Optimismus in fast allen Belangen, seine ansteckende Begeisterung für die wissenschaftliche Arbeit und nicht zuletzt seine bodenständige, ehrliche und freundliche Art mit den Menschen in seiner Umgebung umzugehen.

Ein besonderes Dankeschön geht auch an Ingo Bechmann, der entscheidend zu der Durchführung und Fertigstellung dieser Arbeit beigetragen hat.

Allen Leipziger und Hallenser Laboranten und Laborantinnen, die mir Arbeit abgenommen, oder sie mir erleichtert haben, möchte ich danken. Ein besonderer Dank gilt hier insbesondere Constance Hobusch, Julia Braune und Janine Fröba.

Allen meinen Schul- und Studienfreunden, dir mir lieb und wichtig sind, möchte ich danken, dass sie da sind. Besonderer Dank gilt meinen beiden besten Freunden Moritz Boll und Luis Vollmers, die in Freud und Leid immer bedingungslos an und vor allem auch auf meiner Seite waren und mich immer akzeptiert haben wie ich bin.

Danken möchte ich auch meiner Freundin und Partnerin Johanna Duschek, weil ich keine Gelegenheit auslassen will, ihr zu sagen, wie toll es ist, dass es sie gibt.

Zu guter Letzt möchte ich meinen lieben Eltern danken. Sie haben mir ein Leben voll mit Zuneigung, Liebe und Musik geschenkt und mir ermöglicht, das zu tun, was ich tun möchte und zu entdecken, was das Leben schön und lebenswert macht.